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Adenoid cystic carcinoma of breast: Recent advances

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Key words: Adenoid cystic carcinoma; Breast; Triple-negative and basal-like phenotype; Histology; Molecular genetic features

Core tip: Adenoid cystic carcinoma (ACC) of the breast is a rare, special subtype of breast cancer characterized by the presence of luminal and basaloid cells arranged in specific growth patterns. Although ACCs display a triple-negative, basal-like phenotype, these tumors are usually low-grade and exhibit an indolent clinical behavior. Many discoveries regarding the molecular genetic features of the ACC, including a specific chromosomal translocation t(6;9) that results in a *MYB-NFIB* fusion gene, have been made in recent years. This review provides our experience with ACCs, as well as an overview of its clinical, histopathological, and molecular genetic features.

Abstract

Adenoid cystic carcinoma (ACC) of the breast is a rare special subtype of breast cancer characterized by the presence of a dual cell population of luminal and basaloid cells arranged in specific growth patterns. Most breast cancers with triple-negative, basal-like breast features (*i.e.*, tumors that are devoid of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 expression, and express basal cell markers) are generally high-grade tumors with an aggressive clinical course. Conversely, while ACCs also display a triple-negative, basal-like phenotype, they are usually low-grade and exhibit an indolent clinical behavior. Many discoveries regarding the molecular and genetic features of the ACC, including a specific chromosomal translocation t(6;9) that results in a *MYB-NFIB* fusion gene, have been made in recent years. This comprehensive review provides our experience with ACC of the breast, as well as an overview of clinical, histopathological, and molecular genetic features.

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INTRODUCTION

Invasive breast carcinoma comprises a heterogeneous group of tumors with various clinical, morphologic, and molecular genetic features^[1,2]. According to the 2012 World Health Organization classification, invasive ductal carcinoma of no special type (NST) is the most common histologic type, accounting for up to 75% of all invasive breast carcinomas^[3]. The remainder of the invasive cancers represent at least 18 different special and rare histomorphologic subtypes, including adenoid cystic carcinoma (ACC), a salivary gland-type of breast carcinoma^[3].

A characteristic histologic pattern of ACC of the breast includes both epithelial and myoepithelial compo-

Table 1 Clinical characteristics of adenoid cystic carcinoma of the breast in recently reported cohorts

Ref.	No. of patients'		Pathologic T1 or T2	Lymph node involvement	Distant metastasis	Survival
	Cases	Age (yr)				
Kulkarni <i>et al</i> ^[14]	933	60 (median)	Not reported	5.1%	Not reported	88% (5 yr)
Coates <i>et al</i> ^[15]	376	62 (mean)	90%	6.1%	1.1% (site not specified)	90% (10 yr)
Ghabach <i>et al</i> ^[11]	338	63 (mean)	95%	1.7%	< 1% (site not specified)	94.9% (10 yr)
Thompson <i>et al</i> ^[16]	244	62 (median)	92%	4.9%	2.9% (site not specified)	94.9% (10 yr)
Khanfir <i>et al</i> ^[17]	61	59 (median)	88%	0%	6.5% (bone, liver, lung)	94% (5 yr)
Defaud-Hénon <i>et al</i> ^[18]	30	61 (median)	95%	0%	10% (bone, liver, lung)	Not calculated
Vranic <i>et al</i> ^[19]	21	60.8 (mean)	85%	0%	20% (bone, kidney, lung)	90% (5 yr)

nents and resembles a well-known tumor of the salivary gland origin known by the same name. However, patients diagnosed with ACC of the breast have a better prognosis than those who are diagnosed with ACC of the salivary gland^[4-6]. ACC of the breast belongs to the basal-like subgroup of breast cancers^[7-9]. Based on extensive molecular and genetic profiling studies, basal-like tumors are most often hormone receptor [estrogen receptor (ER) and progesterone receptor (PR)] negative, do not express human epidermal growth factor receptor 2 (Her2), but express one or more basal/myoepithelial cell markers [*e.g.*, cytokeratins (CKs) 5, 5/6, 14 and 17]^[10]. Unlike other triple-negative breast cancers that are associated with poor prognosis, ACC has an overall excellent prognosis^[11]. Because of these distinct clinicopathologic features that set it apart from the other triple-negative breast cancers, an understanding of ACC of the breast is essential for surgical pathologists, breast surgeons, and oncologists. This review will focus on ACC of the breast and will outline important updates in its epidemiology, clinical features, histomorphologic/immunohistochemical characteristics, molecular genetic features, and prognosis/treatment. In addition, we will address our team's experience with this clinical entity.

EPIDEMIOLOGY

ACC is an uncommon subtype of invasive breast carcinoma and accounts for less than 0.1% of all primary carcinomas of the breast^[3,12,13]. Recently, several independent studies based on large patient cohorts have provided more insight into its epidemiology and clinical characteristics^[11,14-20]. This information, in the recent studies published in 2010 and after, is summarized in Table 1. The reported age distribution for patients diagnosed with ACC of the breast ranges from 38 to 81 years (with a median age of 60 years; Table 1) and is similar to that seen in other invasive breast cancer cases^[3]. Moreover, a previous case series of 338 patients with ACC of the breast conducted over a 30-year period identified its age-adjusted incidence ratio (AAIR) to be 0.92 per 1 million person-years. The AAIR remained constant during the 30-year period and was 39%, lower in African-Americans than in Caucasian-Americans^[11]. Most cases are in females, but occasional cases have been reported in male patients^[21,22].

CLINICAL FEATURES

ACC of the breast affects the left and right breasts equally and tumors arise irrespective of the breast quadrants. However, in about 50 percent of patients, lesions are found in subareolar region^[23]. Pain or tenderness described in the minority of cases has not been correlated with histologically-confirmed perineural invasion^[24]. Mammographically, these tumors may appear as asymmetric densities or irregular masses. Sonographically, they appear as well-defined, irregular, heterogeneous, or hypoechoic masses. Nonetheless, the radiographic findings are non-specific and can be misdiagnosed as benign lesions^[13,25]. Subsequently, it could be challenging for a radiologist to make the correct diagnosis of carcinoma without histologic confirmation^[25]. Lastly, although most patients present with a solitary tumor, a few cases of multifocal ACC of the breast have also been reported^[26,27].

HISTOMORPHOLOGIC/IMMUNOHISTOCHEMICAL CHARACTERISTICS

The mean size of ACC is 3.0 cm (range, 0.7 to 12.0 cm)^[28]. Most cases are macroscopically well-circumscribed. Occasionally, pink, tan, or gray microcysts are evident^[28]. ACC usually presents as a localized disease of pathologic T1 or T2 (Table 1).

The histology of ACC of the breast is similar to that of their salivary gland counterparts. A variety of microscopic patterns detected in the ACC of the salivary glands may also be present in the ACC of the breast. A tumor typically consists of a dual-cell population of luminal and myoepithelial-basal cells which may be arranged in one or more of three architectural patterns: tubular-trabecular, cribriform, and solid-basaloid (Figure 1)^[3]. There are two types of structures lined by these two different types of cells: true glandular spaces and pseudolumina. Luminal cells, characterized by round nuclei and eosinophilic cytoplasm, surround true gland lumina containing periodic acid-Schiff-positive neutral mucin. Immunohistochemically, the luminal cells are positive for CK7, CK8/18, epithelial membrane antigen, and CD117 (c-Kit)^[2,29-31]. On the other hand, the myoepithelial-basal cells exhibit central oval nuclei and scant cytoplasm, and form pseudolumina, which result from intraluminal invaginations of

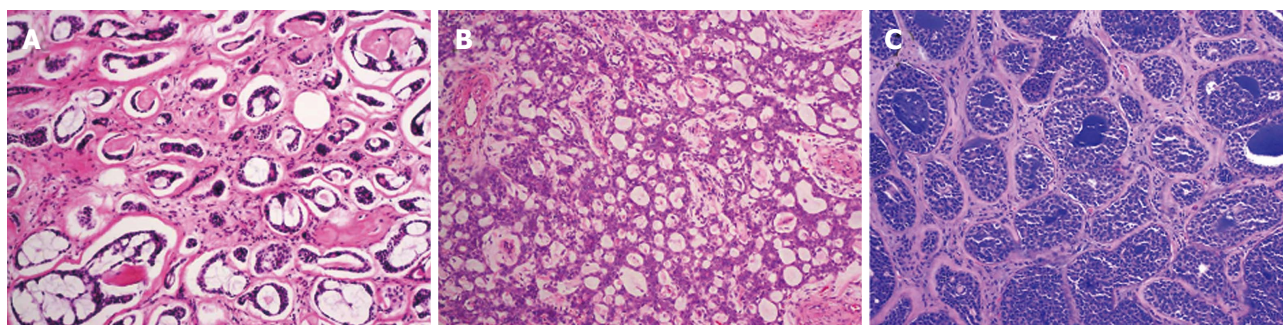


Figure 1 Adenoid cystic carcinoma of the breast. Adenoid cystic carcinomas predominantly showing tubular-trabecular (A), cribriform (B), and solid-basaloid patterns (C). Original magnification $\times 100$.

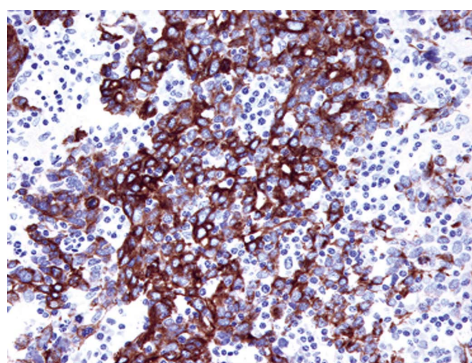


Figure 2 Immunoreactivity of cytokeratin 5/6 in solid pattern of adenoid cystic carcinoma of the breast. The tumor cells are immunoreactive for cytokeratin 5/6, indicating myoepithelial-basal cell origin of tumor cells. Original magnification $\times 200$.

the stroma. The myoepithelial-basal cells are immunoreactive for basal cytokeratins (CK5, CK5/6, CK14, CK17) (Figure 2), myoepithelial markers (p63, actin, calponin, S-100 protein), vimentin, and epidermal growth factor receptor (EGFR)^[2,29-32]. Kasami *et al.*^[33] reported that the polarity of the different types of cells could be demonstrated by immunohistochemistry: myoepithelial-basal cells usually express laminin, fibronectin, basal lamina related proteins, and type IV collagen, whereas the luminal cells express proteins related to cell polarization and epithelial differentiation, including fodrin, E-cadherin, and β -catenin. The authors suggest that this preserved cell polarity and segregated cell differentiation could explain the lack of metastatic capacity observed in this tumor type. Other reports describe areas of squamous differentiation and even rare sebaceous differentiation in ACC of the breast^[34,35].

In a way akin to the ACC of the salivary gland, ACCs of the breast are graded according to the proportion of solid growth: tumors with either cribriform or tubular-trabecular pattern and without solid elements are considered grade I, tumors with $\leq 30\%$ of solid growth are classified as grade II, and tumors having more than 30% solid growth are designated grade III^[4,36]. Ro *et al.*^[4] reported that tumors with a solid pattern (grade II and III) had a tendency to be larger than those without a solid pattern (grade I), and that grade II and III tumors were more

Table 2 Review of data reported on the expression of prognostic and predictive markers of breast adenoid cystic carcinoma (%)

Ref.	No. of cases	Percentage of cases showing positivity		
		ER	PR	Her2
Kulkarni <i>et al.</i> ^[14]	933	15	13	NA
Ghabach <i>et al.</i> ^[11]	338	12	2	NA
Arpino <i>et al.</i> ^[5]	28	46	36	NA
Mastropasqua <i>et al.</i> ^[36]	20	15	10	0
Azouley <i>et al.</i> ^[41]	18	0	0	0
Crisi <i>et al.</i> ^[42]	6	0	0	0
Weigelt <i>et al.</i> ^[43]	4	0	0	0

ER: Estrogen receptor; Her2: Human epidermal growth factor receptor 2; NA: Not available; PR: Progesterone receptor.

likely to develop recurrences. In their series, three patients who developed metastatic ACC had grade II or III lesions. Furthermore, Shin *et al.*^[37] reported 9 cases of the solid (basaloid) variant of breast ACC in which the tumor cells tended to be larger, with hyperchromatic nuclei showing moderate to marked atypia, pleomorphism, and increased mitotic activity. This solid variant of ACC was associated with an aggressive clinical course. However, it is important to note that the histological grade defined by this system did not correlate with disease outcomes observed in two other studies^[34,38]. The most recent American Joint Committee on Cancer staging manual (7th edition) recommends that Nottingham histologic grading be provided uniformly for all breast carcinomas^[39]. Based on this grading scheme, most ACCs would belong to the histologic grade 1 (3 - 1 + 1) or histologic grade 2 (3 + 2 + 1).

Phenotypically, both luminal and myoepithelial-basaloid cells in ACC of the breast are generally negative for ER, PR, and Her2 proteins (Table 2 and Figure 3)^[11,14,40-43]. The immunohistochemical profile of ACC of the breast fits well within that of triple-negative breast cancers with basal-like features. In one study, ER and PR expression was detected in 46% and 36% of ACC cases, respectively^[5]. Although this cohort was one of the larger series of ACCs reported to date ($n = 28$), the cases were collected from different institutions and did not undergo a central review of the diagnosis. Consequently, it cannot be ruled out that a substantial number of these cases were actually invasive cribriform carcinomas with ER and PR immunoreactivity. In addition, it should be noted that

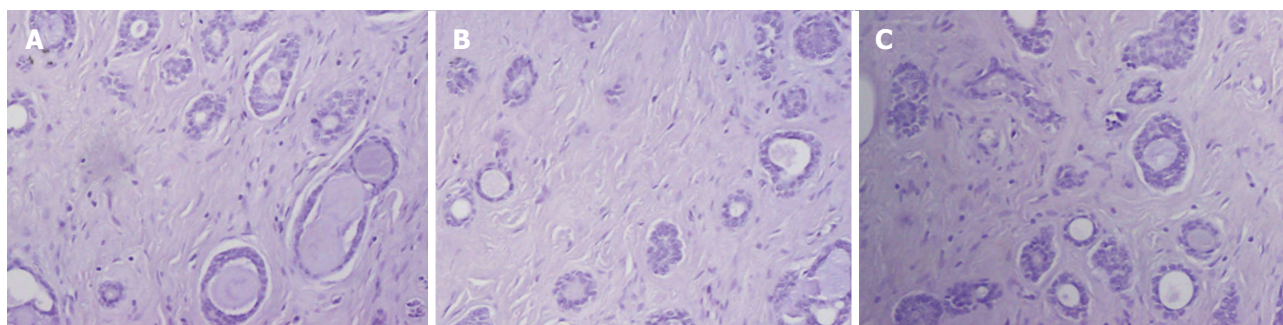


Figure 3 Immunohistochemical findings in adenoid cystic carcinoma of the breast. A: Estrogen receptor; B: Progesterone receptor; C: Human epidermal growth factor receptor 2. All these markers are negative in a case of adenoid cystic carcinoma of the breast. Original magnification $\times 100$.

Table 3 Houston Methodist experience of adenoid cystic carcinoma of the breast (2004 to 2010)

Case No.	Age (yr)	Laterality	Tumor size (cm)	Perineural invasion	Lymph node metastasis	Distant metastasis	TMN stage (AJCC)	Follow-up (mo)
1	61	Left	1.6	-	pN0	-	I A	14
2	83	Right	3.0	-	pN0	Lungs, multiple	IV	85
3	51	Right	2.2	-	cN0	-	II A	12
4	57	Left	4.5	+	cN0	-	II A	65
5	48	Left	2.0	-	cN0	-	II A	90

AJCC: American Joint Committee on Cancer.

in the latter study, dextran-coated charcoal assay was used to assess expression for ER and PR instead of the now more widely used immunohistochemistry. Since normal breast lobules and ducts are often entrapped within the tumor tissues, it may lead to false positive results of the dextran-coated charcoal assay.

There have been several case reports suggesting an association between ACC of the breast and various benign lesions including microglandular adenosis, tubular adenosis, adenomyoepithelioma, and fibroadenoma^[44-48]. Acs *et al*^[44] suggested that ACC of the breast may develop in a background of and in continuity with microglandular adenosis. Following this hypothesis, their group described a morphological spectrum of lesions with a trend of progression, encompassing microglandular adenosis, “atypical microglandular adenosis” (also described as “ACC in situ”), and invasive ACC^[44]. Da Silva *et al*^[45] reported a morphological characterization of tubular adenosis arising concurrently with ACC in the breast, although the comparative genomic hybridization (CGH) analysis performed on these two lesions failed to provide evidence of molecular evolution from tubular adenosis to ACC. Importantly, breast that harbors an ACC can rarely also contain other types of carcinoma, as was shown in a case where the ACC of the breast coexisted with an invasive ductal carcinoma of NST^[49,50].

ACC of the breast that exhibits a cribriform/tubular pattern should be distinguished from invasive cribriform/tubular carcinoma or a benign condition termed collagenous spherulosis^[51,52]. This is especially important when a pathologist is provided with tiny tissue specimens obtained by core needle biopsies^[36]. Invasive cribriform/tubular carcinomas are characterized by the hyper-proliferation of a single type of neoplastic cells (*i.e.*, luminal

cell) only, in contrast to the dual cell types observed in ACC. Moreover, cribriform/tubular carcinomas are generally immunoreactive for ER and PR, whereas ACCs are negative for both^[53]. In addition, limited evidence exists of c-Kit and/or p63 immunoreactivity in ACCs of the breast (positive for both), compared to the invasive cribriform/tubular carcinomas which are negative for both markers^[40]. In collagenous spherulosis, collagenous spherules are irregular, mostly observed at the periphery of the lesions, and no mucosubstance is detected within lumina. Immunohistochemically, ACCs are c-Kit (+), calponin (-), and smooth muscle myosin (-), whereas collagenous spherulosis lesions are c-Kit (-), calponin (+), and smooth muscle myosin (+), which may help to differentiate between these two types of lesions^[54]. The differential diagnosis of the solid (basaloid) variant of ACC includes small cell carcinoma (neuroendocrine carcinoma), solid papillary carcinoma, metaplastic carcinoma, and malignant lymphoma^[37]. Although an extensive and careful search for a more typical cribriform pattern of ACC should be performed, immunohistochemistry can also be helpful to distinguish these tumors from ACC.

MOLECULAR GENETIC FEATURES

Microarray-based gene expression profiling studies have been performed in common types of breast cancer, such as the invasive ductal and lobular carcinomas^[7-9]. However, most of these studies did not focus on special types of breast cancer, and consequently, there is only limited transcriptomic data on the ACC features. A recent molecular subtype analysis using a single sample predictor (*i.e.*, centroid) performed on 4 ACCs revealed that two of the samples were classified as basal-like, while the other two

were shown to exhibit the normal breast-like phenotype. Based on this divergence in the results, they could be an artifact of sample representation, perhaps caused by the contamination with normal tissues^[55]. In fact, molecular subtype assignment following hierarchical clustering showed that all four ACCs consistently displayed a basal-like phenotype, and all of them clustered with one of the five subgroups of the triple-negative breast cancers. In another study that utilized the immunohistochemical staining analysis and microarray-based gene expression profiling for a series of 113 tumors that belonged to 11 special histologic types of breast cancer (including 4 ACCs), Weigelt *et al.*^[43] reported that the ACC, medullary carcinoma, and metaplastic carcinoma were highly similar in their immunohistochemical and gene expression profile. However, ACCs did not intermingle with medullary and metaplastic carcinomas in the hierarchical clustering, but formed a separate group. Another study, an array-based CGH analysis of 59 breast cancers that belonged to 10 special histologic special types established that while medullary and metaplastic carcinomas displayed complex genomes, ACCs consistently exhibited simpler patterns of gene copy number aberrations^[56]. In line with these results, a recent CGH analysis study revealed that ACC of the breast manifested significantly lower frequencies of genetic instability and lower copy number alterations than the histologic grade-matched basal-like and invasive ductal carcinomas of NST^[29]. At the genomic level, ACC is substantially different from the other basal-like breast cancers. Studies show that it rarely harbors genomic aberrations associated with basal-like invasive ductal carcinomas of NST, such as gains of 1q, 6p, 8q, and 10p, and losses of 4p, 5q, and 10q^[29,57,58]. Furthermore, aneuploidy is reported in fewer than 10% of cases with ACC of the breast^[5]. Together, these findings illustrate the heterogeneity of triple-negative, basal-like breast cancers. Although the majority of these tumors are high grade cancers with high levels of genetic instability and an aggressive clinical course (*e.g.*, grade 3 invasive ductal carcinoma of NST, medullary carcinoma, and metaplastic carcinoma), there is also a subgroup of low grade tumors with low frequencies of genetic instability and an indolent clinical behavior (*e.g.*, ACC and secretory carcinoma)^[10,41,43,59-61]. Thus, we emphasize that based solely on molecular subtyping and without proper histologic classification, ACCs, which have an indolent clinical behavior, would be classified as triple-negative, clinically aggressive tumors. Therefore, information regarding the histologic type of triple-negative breast cancers should be included in histopathology reports and taken into account for clinical decision-making.

Although studies using next-generation sequencing (NGS) for whole exome or microRNA expression profiling for ACC of the salivary gland have been recently reported^[62-65], there have been few studies using NGS for ACC of the breast. In one study utilizing microRNA expression profiling for two cases each of ACC of the salivary gland and breast, Kiss *et al.*^[65] reported that the let-7b was overexpressed in ACC of the salivary gland, while

decreased in ACC of the breast. In addition, the miR-24 was decreased in salivary gland-derived but overexpressed in breast-derived adenoid cystic carcinomas.

Similar to ACCs of the salivary gland, ACCs of the breast are characterized by the t(6;9) (q22-23; p23-24) chromosomal translocation, which generates fusion transcripts involving the oncogene *MYB* and the transcription factor gene *NFIB*. Several previous studies reported that this chromosomal translocation is present in over 90% of ACC cases and is a key ACC oncogenic mechanism^[29,66,67]. The myeloblastoma (*MYB*)- nuclear factor I/B (*NFIB*) fusion protein retains the DNA-binding and transactivation domains of a wild-type *MYB*, and is therefore expected to activate *MYB* target genes^[29,66]. *MYB* is a leucine zipper transcription factor that plays an important role in the control of cell proliferation, apoptosis, and differentiation^[68,69], while its target genes include *BCL2* and *GRP78/BIP*, which are essential for cell survival^[70]. *MYB* is a direct target of EG signaling and is highly expressed not only in ACCs, but also in cell lines of ER-positive breast cancers^[71,72]. Recently, one study reported that 67% (8/12 cases) of dermal cylindroma displayed the t(6;9) and *MYB-NFIB* fusion transcripts and that the composition of these chimeric transcripts was identical to that seen in ACC^[73].

Approximately 7% of breast cancer cases are related to hereditary conditions and caused by mutations in the *BRCA1* and *BRCA2* genes^[3]. Although medullary and metaplastic breast carcinomas, with which ACC shares immunohistochemical and molecular findings, show a frequent promotor methylation of *BRCA1* gene, ACC of the breast usually retains normal *BRCA1* gene function^[2,29]. To our knowledge, *BRCA2* gene status has not been investigated in ACCs of the breast.

ACCs of the breast typically do not express the full-length ER- α (ER- α 66) and PR^[11,14,39-42]. However, several studies have shown that the ACC, apocrine carcinoma, and triple-negative breast cancer of NST exhibited a frequent membranous/cytoplasmic immunoreactivity for ER- α 36, a novel ER- α 66 splice variant implicated in membrane-initiated estrogen signaling^[74-76]. In the experimental cell models of breast cancer, ER- α 36 was shown to transduce the membrane-initiated steroid signaling cascade, and served as a dominant-negative modulator of ER- α 66 mediated transcription activity^[75]. In addition, ER- α 36 was reported to be related to non-genomic ER activities, in which activation of the mitogen-activated protein kinase (MAPK/ERK) signaling pathway plays a major role^[75]. The MAPK/ERK signaling pathway is activated in response to antiestrogens (*e.g.*, tamoxifen), indicating a subset of ER- α 66 (-)/ER- α 36 (+) breast carcinomas might still respond to antiestrogen based therapy^[74,75]. Finally, ER- α 36 protein closely interacts with EGFR protein, which is commonly expressed in ACC and triple-negative breast cancers^[75]. Some investigators have reported that ACCs of the breast frequently overexpress EGFR protein in the absence of underlying EGFR gene alterations^[19,29].

Cancer stem cells have been reported to be associ-

ated with tumor initiation, progression, survival, and resistance to therapy^[77]. However, the cancer stem cell field is still fairly controversial and stem cell markers have not been fully elucidated. In the majority of studies, breast cancer cells with a CD44 (+)/CD24 (-) phenotype have been proposed to have tumor-initiating properties with stem cell-like features^[78], and Defaud-Hénon *et al*^[18] recently reported that a characteristic CD44 (+)/CD24 (-) phenotype is commonly observed in the ACC of the breast. On the other hand, frequent overexpression of c-Kit and EGFR proteins was observed in undifferentiated carcinomas with stem cell-like features^[79]. Although several studies illustrated that a consistent c-Kit protein expression was detected in most ACCs^[29,40-43], underlying *KIT* gene alterations, such as gene mutations, have not been previously detected^[80]. Finally, SOX10 transcription factor appears to support stem-like properties in normal tissues and cancer cells^[81]. Recently, Ivanov *et al*^[82] described SOX10 as a novel diagnostic marker for ACCs of the salivary gland and breast basal-like carcinomas, indicating that SOX10 expression might be worth examining in ACCs of the breast.

Although triple-negative NST breast cancers usually have high proliferative activity, ACC of the breast exhibits a low proliferation rate using standard Ki-67 labeling index^[29,83]. Interestingly, their typical proliferation rate is even lower than that of low-grade conventional breast carcinomas^[84]. Mastropasqua *et al*^[40] suggested that proliferative indices showed greater values in high-grade ACCs when compared to low-grade lesions. However, another study reported that the proliferative activity is not associated with the outcome of ACC patients with ACC^[38]. In addition to low Ki-67 labeling index, ACCs of the breast, including high-grade solid-basaloid lesions, also show low p53 protein expression^[29,39,83]. Trendell-Smith *et al*^[53] described a slightly higher p53 protein expression in ACC than that in invasive cribriform carcinoma.

Finally there are several recent studies that identified potential breast ACC biomarkers. Insulin-like growth factor-II mRNA-binding protein 3 (IMP3) is an oncofetal protein and a component of the insulin-like growth factor-II pathway. Studies indicate that it could serve as a biomarker for basal-like breast carcinomas^[84-87], and a recent report showed that the IMP3 is commonly overexpressed in ACCs of the breast^[88]. In another report, the molecular genetic analysis of a primary ACC of the breast and its renal metastasis revealed *PTEN* and *PIK3CA* gene mutations^[89].

PROGNOSIS AND TREATMENT

A striking feature of ACC of the breast, which is in stark contrast with other triple-negative, basal-like breast cancers and the ACC of the salivary gland, is its excellent prognosis. As shown in Table 1, the 10-year survival rate is 90%-100%, and lymph node metastasis is rare, as well as distant metastases, which affect mainly visceral organs^[11,14-19,90]. Based on its indolent clinical course and

favorable outcome, ACC of the breast is generally cured by breast-conserving surgery, such as wide excision or quadrantectomy with or without radiotherapy^[11,17,91]. Mastectomy is recommended for invasive lesions when a cosmetically satisfactory excision is not possible, especially when the tumor has a high-grade pattern^[4,36,92]. A recent study of a large patient cohort reported a considerable benefit of adjuvant radiotherapy on overall and disease-specific survival in patients with ACC^[15]. Moreover, because a high rate of positive surgical margins has been detected following breast conserving surgery, adjuvant radiotherapy may be beneficial^[93]. Furthermore, while some clinicians recommend systemic adjuvant chemotherapy for patients with high-grade lesions or axillary lymph node/distant metastasis^[36], its role in breast ACC patients remains controversial.

When patients with ACC demonstrate local recurrence or distant metastases, a prolonged and indolent clinical course is still likely^[94-97]. However, long-term follow-up is recommended, since their long clinical course carries a risk of secondary malignancies^[98,99], and the risk of distant metastases increases with time^[100].

As treatment of cancer enters a new stage with the development of targeted therapies, the common *MYB-NFIB* fusion gene may provide new therapeutic avenues for the management of advanced ACC of the breast. Consequently, further functional studies investigating the biological consequences of the *MYB* gene of function due to the *MYB-NFIB* fusion are needed. Gene silencing experiments are also necessary to demonstrate that *MYB* expression is required for the survival of cancer cells with genetically activated *MYB*. Finally, the functional role of the ER- α 36 variant in ACC merits further research as experimental evidence in triple-negative breast cancer cell lines suggests that breast cancer cells with ER- α 66 (-)/ER- α 36 (+) phenotype might still be responsive to antiestrogens^[72,73].

HOUSTON METHODIST EXPERIENCE OF ACC OF THE BREAST

A search of the electronic data base at Houston Methodist Hospital from 2004 to 2010 yielded five cases of ACC of the breast. The clinicopathologic and follow-up status of these five patients are summarized in Table 3. The five female patients ranged from 48 to 76 years in age, with a mean age of 60 years. All tumors had distinct morphologic features of classic ACC: histologic grade 1 with cribriform, trabecular or glandular architectural patterns, and basement membrane deposition. No cases of grade II and III tumors were identified. Perineural invasion was identified in one case. Lymphovascular invasion was not seen in any of the cases. An associated adenomyoepithelioma was observed in one case. All patients received lumpectomy and two of these patients had axillary lymph node dissections, with no nodal metastasis found. No patients received adjuvant chemotherapy or radiotherapy. Pulmonary metastasis developed in one case (case 2)

seven years after the initial diagnosis. All of the tumors, including the pulmonary metastatic lesion in case 2, were ER/PR negative and did not express Her2. No synchronous/metachronous in-situ carcinoma, invasive ductal/lobular carcinoma, or microglandular adenosis was reported in any of the cases. Four patients without metastasis were alive and showed no evidence of disease for an average (follow-up) of 45.3 mo (range 12-90 mo). The last patient (case 2) who was diagnosed with pulmonary metastasis is alive with disease at 85 mo (one month after metastasis was detected).

CONCLUSION

The correct classification of the histological special types of breast cancer is not just an academic exercise, as it has both prognostic and predictive implications. Although the majority of triple-negative, basal-like breast carcinomas are high-grade tumors, ACC is a subgroup of low-grade tumors with an indolent clinical behavior that also displays a triple-negative, basal-like phenotype. Because of its low incidence, there have been only few comprehensive studies of ACC of the breast, which is one of the major limitations of this review. However, this review of recent updates, including certain molecular genetic features in breast ACC, herein will hopefully serve as a prognostic and treatment guide for surgical pathologists, breast surgeons, and oncologists, and lead to the development of more specific, personalized therapies for this rare tumor subtype.

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Drug-targeting methodologies with applications: A review

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Abstract

Targeted drug delivery to solid tumors is a very active research area, focusing mainly on improved drug formulation and associated best delivery methods/devices. Drug-targeting has the potential to greatly improve drug-delivery efficacy, reduce side effects, and lower the treatment costs. However, the vast majority of drug-targeting studies assume that the drug-particles are already at the target site or at least in its direct vicinity. In this review, drug-delivery methodologies, drug types and drug-delivery devices are discussed with examples in two major application areas: (1) inhaled drug-aerosol delivery into human lung-airways; and (2) intravascular drug-delivery for solid tumor targeting. The major problem addressed is how to deliver efficiently the drug-particles from the entry/infusion point to the target site. So far, most experimental results are based on animal studies. Concerning pulmonary drug delivery, the focus is on the pros and cons of three inhaler types, *i.e.*, pressurized metered dose inhaler, dry powder inhaler and nebulizer, in addition to drug-aerosol formulations. Computational fluid-particle dynamics techniques and the underlying methodology for a smart inhaler system are discussed as well.

Concerning intravascular drug-delivery for solid tumor targeting, passive and active targeting are reviewed as well as direct drug-targeting, using optimal delivery of radioactive microspheres to liver tumors as an example. The review concludes with suggestions for future work, considering both pulmonary drug targeting and direct drug delivery to solid tumors in the vascular system.

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Key words: Targeted drug delivery; Pulmonary system; Vascular system; Types of drugs and delivery devices; Computational analysis and experimental evidence; Future work

Core tip: Targeted drug delivery to diseased areas or solid tumors has the great potential to significantly improve treatment efficacy, minimize side-effects, and reduce health-care cost. The major problem addressed is how to deliver efficiently the drug-particles from the entry/infusion point to the target site. Past and present developments in drug formulation and associated drug-delivery devices are discussed. Examples of optimal drug delivery to pulmonary target sites as well as targeting solid tumors in the vascular system are reviewed.

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INTRODUCTION

In light of the high cost of medicine and potentially devastating side-effects of drug treatment, targeted drug delivery is of great clinical significance. Thus, targeted drug delivery to solid tumors is a very active research area, focusing mainly on improved drug formulation and associated best delivery methods/devices. Drug-targeting has

the potential to greatly improve drug-delivery efficacy, reduce side effects, and lower treatment costs. However, the vast majority of drug-targeting studies assume that the drug-particles are already at the target site or at least in its direct vicinity.

In this review, drug-delivery methodologies, drug types and drug-delivery devices are discussed with examples in two major application areas: (1) inhaled drug-aerosol delivery into human lung-airways; and (2) intravascular drug-delivery for solid tumor targeting. The major problem addressed is how to deliver efficiently the drug-particles from the entry/infusion point to the target site. So far, most experimental results are based on animal studies.

Concerning pulmonary drug delivery, the focus is on the advantages and disadvantages of the three inhaler types, *i.e.*, pressurized metered dose, dry powder and nebulizer, in addition to drug-aerosol formulations. Computational fluid-particle dynamics techniques and the underlying methodology for a smart inhaler system (SIS) are discussed as well.

Concerning intravascular drug-delivery for solid tumor targeting, passive and active targeting are reviewed as well as direct drug-targeting, using optimal delivery of radioactive microspheres to liver tumors as an example.

The review concludes with future work for both pulmonary drug targeting and direct drug delivery to solid tumors in the vascular system.

PULMONARY DRUG-TARGETING METHODOLOGIES

Targeted drug delivery and controlled release are current challenges in pulmonary drug delivery. Three popular drug delivery ways are per oral (pill swallowing), intravenous (drug injection through the vein), and inhalation (breathing into the human lung).

Pulmonary drug delivery therapies, ranging from the treatment of asthma and chronic obstructive pulmonary diseases (COPD) to lung tumors and systemic diseases, have gained great interest in recent years. Advantages of pulmonary drug delivery, when compared with conventional medical treatments include, improvements in efficiency because of the large surface area of the lung (*i.e.*, 100 m²) and the thin epithelial layer thickness (0.2 to 0.7 μm)^[1], reduction of systemic drug levels with a decrease in adverse effects, and higher degree of convenience^[2-4]. Specifically, as the drug aerosol directly travels to the designated target area, a much lower dose can be used to produce a therapeutic response with negligible side effects^[5].

Pulmonary drug delivery therapies are widely used to treat inflammation, asthma, COPD and cystic fibrosis (CF) as well as diabetes and other systemic diseases. For proper treatment the aerodynamic diameters of drug particles/droplets are recommended to be in the range of $0.4 < d_p < 7 \mu\text{m}$ ^[6,7]. However, due to the sophisticated pharmaceutical aerosol formulations and the complex

anatomy and physiology of human lung airways, the optimization of pulmonary drug delivery (*e.g.*, drug-targeting delivery in lung airways) is challenging. The major research concern in pulmonary drug delivery is on the utilization of physical or chemical mechanisms, novel particles or drug carriers, and new drug-delivery device developments with improved performance.

In this section, three major classes of pulmonary drug delivery systems, *i.e.*, pressurized metered-dose inhaler (pMDI), dry powder inhaler (DPI) and nebulizer, are introduced and discussed, focusing on their delivery mechanisms, efficacies, and challenges for future developments. Furthermore, this section is also devoted to the foundation of lung-aerosol dynamics, *i.e.*, the transport and deposition of particulate drug carriers, which include the parameters affecting drug aerosol transport and deposition characteristics. It will be followed by a description of state-of-the-art methodologies for the realization of optimal pulmonary drug delivery.

Inhalers and drug-aerosol transport

Type of inhalers: There is a rich history of the development of pulmonary drug delivery therapy^[8]. Pulmonary drug delivery devices or inhalers are classified into three major types: pMDIs, DPI and nebulizers^[3].

pMDIs are devices in which the mixture of drugs and propellants is stored in a canister from which accurate amounts can be released when the device is actuated by human power^[3]. Thus, pMDIs provide a fast and cost-efficient solution to deliver pulmonary drug aerosols^[9]. A pMDI expels the drug aerosol driven by propellants, such as chlorofluorocarbons (CFC) which, however, is being phased out due to environmental concerns. More recently, hydro fluoroalkanes propel the medicine through a nozzle at high velocities ($> 30 \text{ m/s}$)^[10]. The typical structure of a pMDI is shown in Figure 1, including canister, metering valve, actuator, and propellant. Detailed functions of each part were presented by Newman^[11].

The advantages of pMDIs are: good portability, accurate dosage control, large capacity of medical doses at low cost^[12]. Disadvantages of pMDIs include: highly dependent on the coordination of the patient's inhalation^[3,13], limited to certain drugs that are physically and chemically inert in the mixture with the propellant, and not efficient to treat deeper lung conditions due to the strong impaction in the upper respiratory system induced by the high jet velocity^[5]. For example, only approximately 10%-20% of the medications emitted from CFC-pMDIs are able to enter and deposit in the lung, while the rest deposits in the oropharynx^[14]. Additionally, the deposition of the content of drug formulation inside the canister can result in an incorrect dose of medication delivery (en.wikipedia.org/wiki/Metered-dose_Inhaler).

To replace CFC and find a new propellant, hydrofluoroalkane was introduced^[12] and approved. To resolve the synchronization problem between device actuation and patient's inhalation, breath-actuated MDIs were developed; for example, the Maxair Autohaler^[15]. The devices actuate early during inspiration at an inspiratory flow rate

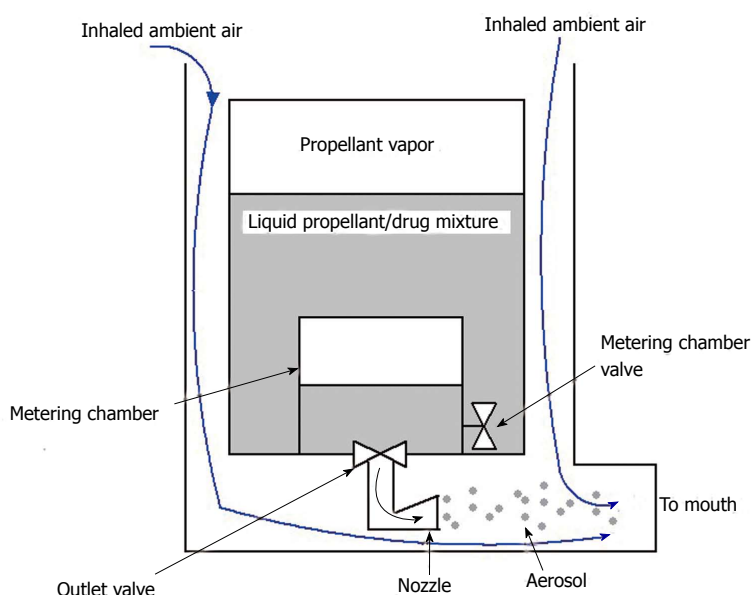


Figure 1 Typical structure of pressurized metered-dose inhaler^[23]. (Reprinted with permission from Ref.^[23]).

of 30 L/min and are well accepted by patients^[12,16]. It is announced that using breath-actuated inhalers might improve asthma control and reduce overall cost of asthma therapy compared with conventional pMDIs^[12,17].

Furthermore, to improve efficacy of drug delivery and avoid synchronization difficulties between patient's inhalation and pMDI actuation, spacer devices have been introduced as an alternative method. The basic design of a spacer contains 3 parts: the open tube, the reservoir/holding chamber, and the reverse flow design in which the pMDI is fired in the direction away from the patient. A one-way valve is used to create a holding chamber after pMDI actuation. Holding chambers serve as particle size filter and produce a fine aerosol because of the stronger inertial impaction of drug-aerosol particles with larger size and particle evaporation of propellant within the chamber. Studies indicated that using a larger-volume (> 750 mL) holding chamber (also called spacer) can provide higher fine-particle doses than a pMDI alone^[18]. However, spacers may decrease the portability of pMDIs.

DPIs are an alternative to pMDIs, delivering pulmonary drugs into the respiratory system in the form of dry solid particles^[3]. The dispersion of a dry powder aerosol is conducted from a static powder bed. When the patient takes a breath, air is introduced into the powder bed which creates turbulent flow and leads to fluidization of a static powder blend, entering the patient's airways. DPIs contain medicine in a variety of types, *e.g.*, single-dose capsule-based designs, multi-dose units containing the drug in bulk, and multi-dose units containing individual blister packages^[19,20]. DPIs can also be divided into two types in terms of its drive force, *i.e.*, a passive DPI dependent on patients' inhalation and an active DPI dependent on external forces^[21,22]. Active DPIs have become a preferred method to uniformly distribute drugs independent of the inspiration flow. A typical structure of a unit-dose DPI is shown in Figure 2.

DPIs are small, portable and easy to use. Several advantages were discussed by Sahane *et al.*^[3]. For example,

there is no need for coordination of actuation and inhalation, because the patient's inspiratory force de-aggregates the powder and generates the aerosol. Furthermore, DPIs are able to deliver higher drug payloads to the airways^[20].

However, there are several disadvantages of DPIs. For example, if the therapeutic dose of a drug is high, the patient needs to manually reload several individual units per dose, as delivery is limited to a capsule-based or blister-based unit^[19]. Also, DPI medications must be stored in a dry place at a temperature of not more than 25 °C and humidity between 40%-50% in sealed packages (en.wikipedia.org/wiki/Dry_Powder_Inhaler). Specifically, exposing the powder in a high-humidity environment destroy the medication dispersion ability of the device, implying that the efficacy of a DPI depends mainly on the flow properties of the powder suspension.

Nebulizers are breathing devices that generate droplets in small scales from a liquid in solution/suspension^[12], which are used to treat lung diseases. The inhaled drug is in the form of mist aerosols. Nebulizers are often used in situations in which a conventional inhaler is ineffective. In simple targeting cases, nebulizers may limit side effects of certain medication, say, steroids, by delivering the drug directly to the desired site. Several conventional designs of nebulizers are shown in Figure 3.

Atomizers (or jet nebulizers)^[23] are the most common ones (Figure 3A). They use compressed gas, or a compressor, to generate high-velocity air streams through a tight opening and across the fluid medication suspension to create particulate/droplet aerosols. The fluid is split up by the airstream and divided into droplets inside the nebulizing chamber. The primary advantage of jet nebulizers is the low operational expense. However, there is always the lack of portability because of the need of compressed gas^[12].

Ultrasonic wave nebulizers generate aerosols *via* the vibration of a piezoelectric crystal at a high frequency (> 1 MHz) through the drug liquid (Figure 3B). Contrasted

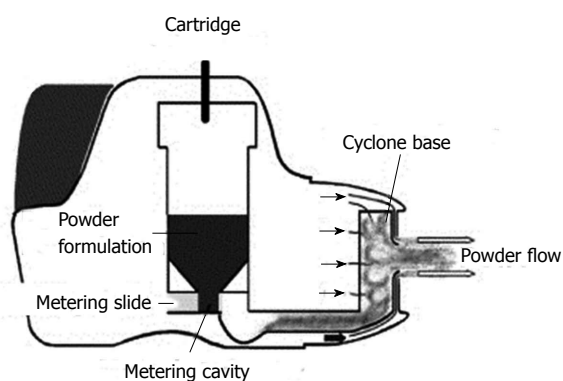


Figure 2 Typical structure of unit-dose dry powder inhaler.

with jet nebulizers, ultrasonic nebulizers work with lower noise and provide faster delivery of pharmaceuticals; although due to the complimentary heat generated during the operation, there are several medication restrictions for the ultrasonic wave nebulizers. With both types of nebulizers, the patient inhales vapor through a mouthpiece or face mask. Electronic nebulizers form a subcategory of ultrasonic nebulizers.

Based on the vibrating mesh technology (VMT), smaller portable devices have been developed as advanced nebulizers, *i.e.*, vibrating mesh nebulizers^[24]. With the VMT, a mesh/membrane with multiple apertures vibrates at the top of the liquid reservoir (Figure 3C), generating drug aerosols consisting of small-scale droplets. Vibrating mesh nebulizers are claimed to have higher output efficiency, minimal residual volume, and high percentage of fine particulate drugs in the emitting stream^[24].

Another type of new-generation nebulizers is called human-powered nebulizers which are breath-enhanced^[25]. Their improved designs avoid exhaled loss and apparatus loss of aerosols. Ambient air is entrained through a one-way valve along with the power gas during inspiration, while during exhalation the one-way plastic flapper valve is closed.

As part of the developments of human-powered nebulizers, a dosimetric nebulizer is defined as one that releases aerosols only during inhalation, being the most efficient nebulizer generating aerosols. Comparisons of different types of nebulizers are presented in Table 1^[12]. A variety of nebulizer products on the market are summarized in Table 2.

In addition to the three major classes of inhalers, new types of inhalers were designed in order to improve the efficacy. "Soft mist inhaler" is another type of drug delivery device^[5,26]. It was developed in order to overcome the limitations of traditional inhaler devices and to meet the need for a convenient propellant-free inhaler^[5]. For example, the Respimat® Soft Mist™ inhaler (SMI) utilizes the mechanical force from a spring instead of a fluid-gas propellant to produce a drug aerosol which is suitable for inhalation. The spring system inside the inhaler can guarantee that the aerosol is produced by a reliable and reproducible energy source. Thus, dosage and size distribution of the drug aerosols are insensitive to the

Table 1 Comparisons of conventional categories of nebulizers

	Jet	Ultrasonic	Vibrating mesh
Features			
Power source	Compressed gas or electrical mains	Electrical mains	Batteries or electrical mains
Portability	Restricted	Restricted	Portable
Treatment time	Long	Intermediate	Short
Output rate	Low	Higher	Highest
Residual volume	0.8-2.0 mL	Variable but low	< 0.2 mL
Environmental contamination			
Continuous use	High	High	High
Breath-activated	Low	Low	Low
Performance variability	High	Intermediate	Low
Formulation characteristics			
Temperature	Decreases	Increases	Minimum change
Concentration	Increases	Variables	Minimum change
Suspensions	Low efficiency	Poor efficiency	Variable efficiency
Denaturation	Possible	Probable	Possible
Cleaning	Required, after single use	Required, after multiple use	Required, after single use
Cost	Very low	High	High

inspiratory characteristics of the patient. Medicine delivered by the SMI is stored in a collapsible bag in a sealed plastic container inside the cartridge. With each actuation, the correct dose is drawn from the reservoir and the flexible bag contracts, which is launched by the spring^[27]. A recent papers^[28] also claimed that SMI can avoid the coordination difficulty between inhalation and actuation. Also, the emitted stream velocity of the aerosol is much slower than that of a pMDI. Theoretically, with the SMI aerosol transport to deeper lung airways is easier due to lower inertial impaction in the upper respiratory system, so that the resulting deposition fraction can reach 40% for adults^[19]. However, besides the advantages of SMI, such a device is relatively expensive. Other SMI designs include AERx, and AERx Essence platform (Aradigm, Hayward, California)^[29].

MDIs and DPIs are both portable and fast delivering devices for low medication dosages. Another characteristics of both device types is that the aerosol generator and the medication are not detachable. In contrast, nebulizers are able to deliver high medication dosages, and a single device can be used with different drugs^[30].

Compared to pMDIs and DPIs, another advantage of using nebulizers for drug inhalation is that no special inhalation techniques are required^[12]. However, due to the need of compressed gas or a compressor to operate, conventional nebulizers are generally not portable. Additionally, the drug-delivery efficacy and treatment time using conventional nebulizers are much lower and longer than those for pMDIs and DPIs^[31]. However, presently there appears to be a tendency among physicians to prefer to prescribe a pMDI rather than a jet nebulizer which produces more noise and is less portability.

Comparative efficacy of different pulmonary drug delivery devices has been performed by different research groups. Chou *et al*^[32] concluded that MDIs can give a

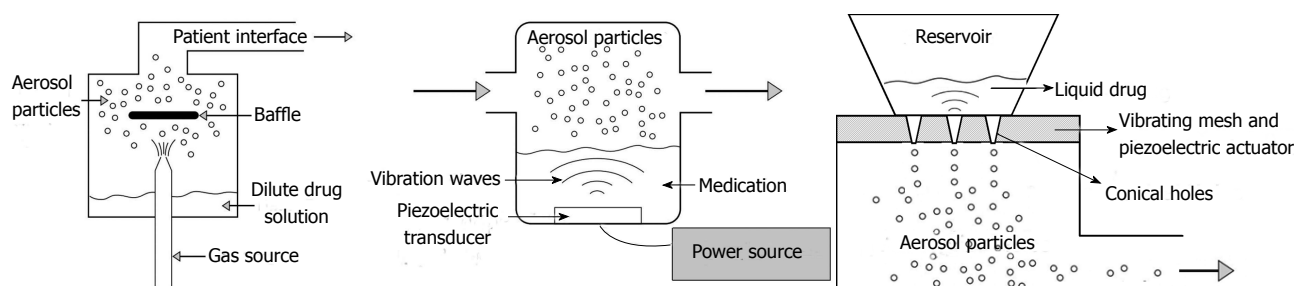


Figure 3 Typical structures of different nebulizer categories. (Reprinted with permission from Ref.^[117]. A: Atomizer (jet nebulizer); B: Ultrasonic wave nebulizer; C: Vibrating mesh nebulizer.

Table 2 Advantages and disadvantages of different nebulizer products

Product name	Type	Pros	Cons
PARI Vios®	Jet nebulizer	Low operational cost, robust in structure, effective in nebulizing suspensions	Relatively bigger in size and heavier in weight; more noisy
Omron MicroAir®	Electronic nebulizer	Does not heat the medicine, quiet and fast drug delivery	Comparatively expensive; replaced much more frequently
Omron NE-U17®, Beurer Nebulizer IH30®	Ultrasonic nebulizer	Silent and portable	
Omron NE-U22V®, Pari E-Flow®	Vibrating mesh nebulizer	Higher output efficiency, minimal residual volume, and High percentage of particles in the emission, small in size	Less efficient in nebulizing aerosols
Pari LCD®	Breathe-enhanced nebulizers	Higher output efficiency avoiding apparatus loss and exhaled loss	N/A
AeroEclipse®	Dosimetric nebulizer	Higher output efficiency avoiding apparatus loss and exhaled loss	N/A

faster and more economical approach to deliver bronchodilator drug aerosols for asthma treatment in elder children and adults. A similar conclusion was reported by Batra *et al.*^[33] for the aerosolized salbutamol in an acute exacerbation of asthma in children. Delgado *et al.*^[9] investigated nebulizers *vs* MDI with spacers for bronchodilator therapy to treat wheezing in children aged 2 to 24 mo. They drew the conclusion that although younger patients are often unable to coordinate inspiration with activation of the MDI, thereby limiting the effective amount of drug inhaled, MDI with spacers may be as efficacious as nebulizers for the emergency department treatment of wheezing in children aged two years or younger. Dhuper *et al.*^[34] claimed that using a MDI with a spacer may result in a marked reduction in time and effort, thereby saving the total cost for treatment compared with conventional nebulizers.

While the optimization of treatment using MDIs requires coordination between inspiration and actuation, which is difficult to achieve by patients^[13], most DPIs generate and deliver drug aerosols depending on subject-specific inhalation efforts. Indeed, the dose delivered by DPIs is more related to the inspiratory flow rate than by the device^[35]. Specifically, MDIs are able to generate more consistent aerosol sizes across a range of inspiratory flow rates. In contrast, the aerosol-size distributions produced by DPIs are reported to be highly dependent on the inhalation patterns, with some being showing more sensitive than others^[36].

Not surprising, all drug delivery devices have advantages and limitations. Device selection must consider portability, convenience level for users, therapeutic efficacy

as well as low cost and high safety^[12]. As mentioned, the therapeutic efficacy is determined by many factors, *i.e.*, the kind of medication used, the aerosol's physical and chemical properties, as well as the patient's inhalation patterns and the physiology of their respiratory systems^[30].

The efficacy enhancement of drug delivery devices can be achieved through two major strategies: (1) improved drug formulation; and (2) improved device structure design. The primary goal is to control the agglomeration of drug aerosols inside devices to reduce drug residues in the device, thereby enhancing the efficacy.

Drug formulation: The physical and chemical properties (*e.g.*, particle size distribution, shape, surface charge, hygroscopicity, *etc.*) of drug-particles are most important. Usually, drug formulations, including drug-carrier selections, are able to lower inhaler retention and improve transport, the proportion of drugs that reach the desired lung site, and the stability of the drug *in vivo*. Sufficiently strong attractions between carrier and drug must be guaranteed for maintaining the stability of the medication mixture. However, the attractions between carrier and drug must also be sufficiently weak so that the release of the medication from the carrier will not be impeded inside the human respiratory system^[3]. Sahane *et al.*^[3] also discussed the formulation of drugs for DPIs. For example, DPIs are formulated using four types of formulation strategies: Carrier Free, Drug Carrier, Drug Additive, Drug Carrier Additive. Specifically, therapeutic dry powders for inhalation are often composed of fine drug particles and inert coarse carrier particles such as lactose^[37].

Sufficient detachment of the drug from its carrier must be guaranteed to improve the delivery efficiency. Improved formulation should reduce the particle-particle interacting forces which are the major cause of the agglomeration between drugs and carriers. Furthermore, adding preservatives and mixing it with other drugs will also influence device output and aerosol characteristics in a good way^[38]. Formulations for pMDIs are also necessary. For example, mast cell stabilizers such as cromoglicate or nedocromil are used to extend the duration of the device^[11]. In the recent decade, non-hygroscopic mannitol exhibits particularly great promise as an attractive carrier in DPI systems to replace lactose^[37,39]. Additionally, it is necessary to minimize the adhesion intensity between the drug particle and the inner wall of the inhalers by using surface treatment to reduce inhaler retention and enhance the drug delivery efficacy^[40].

Device structure: Device structures can have a strong impact on the velocity field inside the device, thereby influencing the drug suspension characteristics. For example, the design of the actuator plays an important role in the delivered spray characteristics generated by pMDIs^[11]. Specifically, the design variables of the actuator are expansion chamber size and shape, nozzle diameter, nozzle path length, mouthpiece length and shape, breath-actuation, spray velocity modification, and spacer attachment^[19,41]. For nebulizers, the chamber design using a one-way valve is important to decrease aerosol waste during exhalation^[27]. Also, the mouthpiece design is essential to lower the percentage of drugs remaining in the device. For example, increasing the cross-sectional area of the mouthpiece section will decrease the emitting velocity of the drug aerosol thereby lowering the extent of drug deposition in the oral cavity due to the inertial impaction effect^[42]. Other factors related to structure which will influence the device performance are flow path design and manufacturing design^[7].

The patient's inhalation behavior, in light of the correct utilization of a drug delivery device, is likewise essential for successful therapy. Specifically, it needs to be guaranteed that a patient can easily use the device correctly and properly thereby optimizing the therapeutic efficacy^[27]. Therefore, the ideal design of drug delivery devices should include the following characteristics^[11,22]: (1) attractive in appearance, easy to use, and easy to carry; (2) accurate and uniform delivery of medication over a variety of patient's inhalation intensities; (3) reliable qualitative drug delivery control throughout the life of the inhaler; and (4) low cost with high efficacy.

Traditionally, the biotechnology and pharmaceutical industries preferred jet nebulizers for novel medication development, considering their capabilities to deliver higher doses of drugs and the lower research and development expenses than other types of devices^[19]. However, recently many companies have recognized the long time duration at each treatment experienced by patients. Therefore, to handle that issue they have been starting preclinical development with novel devices based on more efficient and advanced drug delivery technologies.

In the previous two decades, a few innovative inhaler designs have been developed, providing more efficient drug aerosol delivery^[12]. New designs and new improvements were presented in several papers^[11,12,19,20,43-46]. For example, the improvement of pMDIs is mainly focusing on providing more precise targeting, dose metering, and easy actuation. Related new products are listed by Dolovich *et al.*^[12]. For new designs of the major three classes of inhalers, Zhang *et al.*^[20] proposed novel active and multi-dose dry powder inhalers which are able to utilize the compressed air to deliver a small quantity of extra fine particles with high delivery efficacy. A new design for a nebulizer with flow meter function was proposed by Addington *et al.*^[46]. By changing DPI device structure and drug formulation, Behara *et al.*^[47] proposed a design option of a DPI which controls the aerosol diameters and increases the emitted drug dose.

Drug-aerosol dynamics: Liquid and solid micro/nano-particulate matter (*i.e.*, solid particles and droplets) and vapors are generated by drug delivery devices for therapeutic purposes. The objective of targeting is to guide and deliver drugs from their releasing position to expected deposition regions in the human respiratory system to optimize the medical effectiveness^[48]. The fluid-particle dynamics during drug-aerosol transport, deposition, absorption, and clearance are essential for guiding the optimization technique of drug delivery. Specifically, "targeting" can be categorized into three levels^[1]: (1) delivery to a specific lung region, *i.e.*, central or peripheral, right or left; (2) delivery to the site of disease; and (3) delivery to distinct cell types with biological barrier transport, *e.g.*, epithelial cells, or cells of the lung associated lymphatic tissue. Traditional targeting activities can be also grouped into passive and active targeting^[49].

As discussed in the previous sections, great progress has been made in drug aerosol formulation for better drug-aerosol delivery efficiency, as well as controlling drug aerosol transport before deposition in lung airways. However, it is also necessary to understand the absorption and systemic transport of drug aerosols after the deposition at the air-blood barriers, which has been rarely investigated. In this section, underlying principles for drug-aerosol transport and deposition in human respiratory systems are discussed to provide valuable insight into certain aspects for optimal pulmonary drug-targeting.

Physical and chemical factors which can affect the transport and deposition of drug-aerosols include: initial particle size, shape, density, concentration, as well as release position and velocity; furthermore, the drug-aerosol formulation (*i.e.*, hygroscopicity, charge and surfactant) as well as the geometric characteristics of the patient-specific respiratory system are important^[6,41,48].

The underlying principles to describe the complex and coupled fluid-aerosol dynamics inside the human respiratory systems are the physical conservation laws, using the Eulerian or Lagrangian modeling approach. Specifically, the Eulerian approach can be employed to calculate continuity, momentum and energy equations

for the continuous airflow phase, while both Eulerian and Lagrange approaches can be used for the discrete drug aerosol phases^[6,48]. Supplementary equations may be necessary for the description of complex mechanisms, *e.g.*, evaporation or condensation of aerosol droplets^[50], magnetic-force driven drug-targeting delivery^[51], rotational motion of non-spherical particles^[52], *etc.* Several computational fluid-particle dynamics (CF-PD) models are available for the calculation of air-drug mixture dynamics^[53], such as the discrete phase model (DPM), two-fluid model, mixture model, dense dispersed phase model (DDPM), and the discrete element method (DEM).

The deposition mechanisms of aerosol particles or droplets - impaction, sedimentation, diffusion and combinations in the respiratory tract - have been extensively discussed^[6,48,54]. Specifically, for microparticles, the deposition may occur due to: (1) secondary airflow (laminar or turbulent) induced wall impaction; (2) inertial wall impaction; (3) gravity induced sedimentation; (4) particle-particle interaction induced wall impaction; and/or (5) diffusion. For nanoparticles, diffusion, caused by Brownian motion, may become most significant.

Other than treating local diseases in the respiratory system, pulmonary drug delivery is also promising for systemic drug delivery which is intended to utilize the alveolar region to swiftly absorb drug aerosols. However, the processes that take place after an aerosol particle has landed on the pulmonary epithelial surfaces, *i.e.*, the dissolution, absorption, mucociliary clearance, and systemic translocation, still lack information^[55]. Using complex pharmacokinetic modeling will be helpful in understanding the absorption process through the lung epithelium, including paracellular transport and transcellular transport. Considering numerical modeling of this process, mass transfer advection-diffusion equations can be employed with proper boundary conditions^[56]. Furthermore, systemic drug transport can be modeled using multi-compartment mass transfer models for drug-aerosol migration into the mucus-tissue-blood system^[57,58]. Factors affecting drug absorption are physiochemical properties of drug aerosol particles (*e.g.*, hydrophobicity), co-administration conditions, lung pathophysiology, *etc.*^[55].

With decreasing computational limitations and the advancements in commercial CFD software development, more realistic numerical models are becoming available for the simulation of dense particle suspensions as well as droplets with heat and mass transfer. Those models can be used for the design of drug delivery devices^[59]. Although experiments can provide some information about the airflow field of the drug delivery device^[60], using CFD-techniques provide more detailed information when compared to experiments. Specifically, computer simulation models require initial and boundary conditions as well as the geometry of the flow domain of the device. Once fully validated, they are cost-effective tools to analyze factors influencing the performance of drug delivery devices, *e.g.*, turbulence in the inhaler, spray momentum and inlet jet effects, as well as best possible geometric de-

sign and operation.

Originally, CFD was only utilized for airflow field analysis of the drug inhalers^[61,62]. With the development of numerical multiphase flow methods, simulation techniques were used for the design and analysis. For example, Kleinstreuer *et al.*^[63] numerically investigated the transport and deposition of drug droplet aerosols from a pMDI into a model of the human respiratory system. The parametric analyses with different propellants, nozzle diameters, and releasing positions were presented. Based on the numerical results, they found that using a smaller nozzle provides a better atomization effect and finer droplets with more uniform dispersion.

Recently, based on the numerical model established by Worth Longest *et al.*^[64,65], Longest *et al.*^[13] investigated different aerosol deposition in human lung airways between a specific MDI and DPI. Fluent 12.0 with user-defined functions (UDFs) was employed for the Euler-Lagrange numerical simulations. They claimed that for the specific inhaler models they investigated, MDI is able to deliver significantly more drugs to the tracheobronchial region when compare to DPI. It is worth mentioning that they did not consider any particle-particle interaction effects. Jiang *et al.*^[66] investigated the design impact on a commercial DPI using the LRN κ - ω model in ANSYS Fluent 6.2.16. However, they did not simulate powder transport and subsequent deposition by using any multiphase flow model. Based on CFD analysis, Longest *et al.*^[67] evaluated associations between aerodynamic parameters and DPI performance for a carrier-free formulation, forming micron/submicron-scale drug aerosols. Factors which may influence the dispersion of aerosol particles were discussed.

With the development of computational fluid dynamics-discrete element method (CFD-DEM), the discrete element method is a robust and computational economic model to simulate the highly dynamic process (*i.e.*, particle-particle interaction) for dense powder dispersions in inhalers^[68]. Inhaler developments based on CFD-DEM simulations have been focused on pharmaceutical agglomerate break-up in DPIs^[59]. For example, Tong *et al.*^[68,69] recently employed ANSYS Fluent with in-house UDFs, describing powder dispersion in a commercial Aerolizer® Inhaler model. They also investigated the factors influencing the performance of the inhaler based on their numerical simulation results. They found that at low flow velocities, agglomerates consisting of particulate matters with smaller diameters were more difficult to disperse. They also claimed that the dispersion efficiency is proportional to the ratio of the particle impact energy and particle-particle cohesion energy.

In summary, it is promising to use CFD-DEM or DDPM-DEM for the simulation of dense drug-powder suspensions in pMDIs and DPIs, because of the DEM capability of taking into account the particle-particle contact interaction as well as the computational economy aspect. CF-PD models can also provide guidance for drug delivery and hence enhancing methodology developments which are discussed in Section 2.2. For a recent review see Ruzzycki *et al.*^[70].

Design and strategies for direct drug-targeting

Existing drug aerosol delivery devices, including those that attempt to target specific areas in the lung, exhibit poor efficiencies (*e.g.*, from 5% to 20%). Efforts are being made to improve direct drug delivery through the pulmonary route. The goal is to provide high doses of drugs to lung tumor tissue *via* inhalation, resulting in treatment efficiencies and low adverse side effects.

Smart inhaler system methodology: Kleinstreuer *et al.*^[63] analyzed computationally the performance of pMDIs with and without spacers and compared their deposition efficiencies with that of a smart inhaler system (US Patent 7900625 issued 03/08/2011) based on a new optimal targeting methodology^[71]. A novel smart inhaler system (SIS), which achieves up to 85% drug-aerosol deposition efficiency, is being prototyped and experimentally tested. The SIS is a device for directed aerosol delivery to predetermined lung sites, facilitated by an adaptive nozzle and a mechanism for inhalation waveform modulation. The aerosol particles are released through a nozzle which incorporates lightweight, multifunctional shape memory materials that allows to move the nozzle's optimal radial position based on subject-specific numerical data. The SIS is promising to notably improve the aerosol delivery efficiency to specific locations through pulmonary routes, thereby reducing unwanted deposition in healthy lung airways.

Enhanced deeper lung delivery of nano- and micro-pharmaceutical aerosols *via* condensational growth:

Drug aerosol losses occur because of high deposition in the nasal passages or in the oral cavity due to impaction. To enhance drug-aerosol delivery into deeper lung airways, enhanced condensational growth (ECG) and excipient enhanced growth (EEG) methods have been proposed and validated by experiments *in vitro*^[13,72-74]. Based on the fact that the larger mass mean aerodynamic diameter of drug aerosols indicates strong impacting, deposition of particles before entering the trachea as well as most submicron particles inhaled will be exhaled without depositing in the lung airways. Thus one can utilize the high relative humidity of the ambient air or inside the human respiratory system to control the trajectories and depositing sites of the particles in human pulmonary routes, relying on different condensational growth rates of such sub-micron aerosols of different formulations. Specifically, submicron particles are emitted from the inhalers which are able to initially penetrate through the oral or nasal cavity, thereby reducing the deposition before entering the trachea. With the condensation effect, those particles will grow in size and most of them will deposit in deeper lung airways. For ECG, the sub-micron aerosols are inhaled with highly humidified air at a temperature higher than that of the human body. The droplets will grow due to the condensation of surrounding water vapors when they enter the human respiratory system^[72]. For EEG, hygroscopic excipients are formulated with the drug and the formulated drug aerosol will absorb water inside the human respiratory system. Although these

methods result in higher pulmonary deposition, they are not able to provide location-specific delivery (see Level 2).

Magnetic nanoparticles for site-specific pulmonary drug delivery:

Another active targeting strategy is magnetic targeting, which can be realized by combining magnetic nanoparticles (*i.e.*, γ -Fe₂O₃ and Fe₃O₄) with micron particles or droplets^[51,75-79]. These types of particles are also called magnetic nano-in-microparticles (NIMs). An external magnetic field will be enforced to guide drug aerosols to specific regions of the lung (Figure 4), thereby reducing undesired side effects, *e.g.*, mitigating deposition on healthy lung tissues. To succeed in direct drug delivery, those magnetic nanoparticles should have characteristics such as mono-dispersity, superparamagnetism, stability and biocompatibility^[80].

However, further translation of magnetic nanoparticles may cause potential health problems and need further clinical investigations. The long-term effects of magnetic nanoparticles need to be studied as well^[77]. For example, concerns associated with long-term tissue damage, toxicity, carcinogenesis, immunogenicity, and inflammation need to be investigated to improve the production of magnetic nanoparticles^[81].

Shape engineering for novel drug carriers: For pulmonary drug delivery, the deposition pattern and clearance from deposition sites are two key parameters for a proper design of drug-delivery carriers^[82]. For example the particle shape of drug carriers has a profound impact on optimizing performance of drug delivery. Compared to spherical particles, numerical studies have shown that fiber-like particles are more likely to reach the deeper lung airways^[52,83]. Also, fiber-like carriers have better internalization abilities than spherical particles for drug delivery^[84]. This finding demonstrates that when targeting drugs into deeper lung airways, fiber-like drug carriers may perform more efficiently than spherical ones. A recent study demonstrated that using elongated fine mannitol particles enhance the aerosolization performance of inhalable drugs which may improve the efficiency of drug delivery from the devices^[37,85]. It is promising to explore the shape as an important parameter for improved drug delivery performance.

Multifunctional Nanoparticles: Today, the size of drug aerosol particles can be reduced from tens of micrometers to tens of nanometers (*i.e.*, less than 100 nm in size), which is a significant technological and medical breakthrough. Drug-delivery systems for nanoparticles have been developed which can potentially enhance the efficacy and reduce side effects for a wide range of drugs. Due to the small inertia of nanoparticles, they can avoid impacting the oral cavity when being inhaled, and hence they are transported deeper into human lung airways. Those nanoparticles with diameters around 50 nm have been reported to be most efficiently internalized by cells^[86]. The design of multifunctional nanoparticles for treatment of pulmonary diseases (*i.e.*, lung cancer) is also

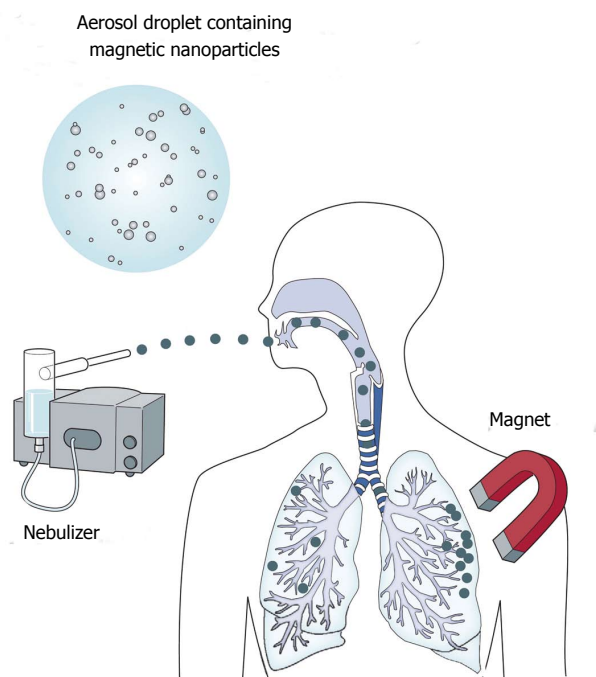


Figure 4 Targeted deliveries of magnetic aerosol particles. (Reprinted from Ref.^[12], with permission from Elsevier).

a promising methodology. Additional functionality such as image contrast enhancement can be realized by adding other constituents into multifunctional nanoparticles, thereby assisting better drug-targeting control. Multifunctional nanoparticles can be designed for detecting infected cells, and delivering drugs specifically to those cells, and leaving healthy organs and lung cells unaffected^[87]. Multifunctional nanoparticles can also deliver multiple therapeutic agents in a single formulation^[49]. A multifunctional nanoparticle consists of a surface coating, imaging agent for detection, and therapeutic drugs. The aims of multifunctional nanoparticles are^[49]: (1) enable specific targeting and aid in uptake realized by surface modification; (2) avoid fast endothelial system clearance *via* surface coating technology^[88], and thereby extending circulation time and enhance uptake; and (3) load higher concentrations of multiple remedial agents that can override multidrug resistance and result in therapeutic effects.

However, more work is needed to understand the fate of nanoparticles after inhalation, including interactions with biological cells and nano-toxicity. Also, in a recent review article, Cheng *et al.*^[89] discussed the costs and regulatory hurdles for using multifunctional nanoparticles *vs* their potential benefits.

INTRAVASCULAR DRUG-TARGETING METHODOLOGIES FOR SOLID TUMORS

In addition to drug-targeting in the pulmonary system, much focus has been placed on treating unresectable tumors *via* intravascular therapies. These therapies involve either systemic or local, intra-arterial delivery of therapeutic agents such as chemotherapeutic drugs, multi-

functional nanoparticles (NPs), radioactive microspheres, or embolic agents. While micron-sized agents must be delivered locally due to their embolic potential, this local drug administration is also beneficial for delivering higher therapeutic concentrations to cancer cells.

Intra-arterial delivery is often achieved using a locally placed drug-infusion catheter. However, due to the often tortuous and small size of the complex arterial systems leading to tumors, it is difficult or impossible to manually position this catheter directly in tumor-supplying arteries. As a result, therapeutic agents can still deposit in healthy tissue and/or travel to other organs. Thus, techniques are needed to better target predetermined cancer sites from upstream. Some existing methods include passive and active targeting for multifunctional nanoparticles as well as magnetic drug targeting. As will be discussed in the next sections, the shortcomings of these methods are that the nanoparticles must be very close to the tumor for passive and active targeting to be effective, and the magnetic particles must be near the body's surface and in slow blood-flow systems for magnetic drug targeting to be successful. As a result, a direct drug-targeting strategy has been proposed which uses knowledge of the patient-specific, local blood flow field to precisely position a smart micro-catheter radially and deliver the therapeutic agents to the tumor directly. Current research is focused on developing and testing such a device.

Passive and active targeting

Being less than 1 μm in at least one dimension, multifunctional nanoparticles can more readily extravasate through tumor vessels and attach to cancer cells with the help of passive and active targeting. Specifically, passive targeting takes advantage of the leaky walls and poor lymphatic drainage of many tumor vessels. This allows NPs to more readily enter the tumor interstitium and linger for extended periods (*i.e.*, the enhanced permeability and retention effect)^[90-100]. Active targeting can then enhance tumor accumulation through ligand-receptor binding which is achieved by incorporating ligands on the drug's surface to selectively attach to over-expressed antigens or receptors on tumor cells^[101,102]. This targeting can also enhance therapeutic efficacy through receptor-mediated endocytosis.

The limitation of these passive/active-targeting events is that the NPs must come in close proximity to the tumors for both strategies to be effective. Thus, the NPs' size, shape, surface properties, and targeting ligands have been modified in attempts to lengthen their circulation time and increase site specific accumulation^[91,103-105]. For example, drug-loaded nanoparticles are often coated with polyethylene glycol to minimize the attraction of proteins which trigger immunogenic responses leading to system clearance. However, while such characteristics are beneficial for prolonging the NPs circulation time, they can also be detrimental once the NPs are near the tumor cells due to their resistance to endocytosis. Thus, the possibility of dynamically altering these characteristics *in vivo* is currently being investigated^[106]. Such functionality has

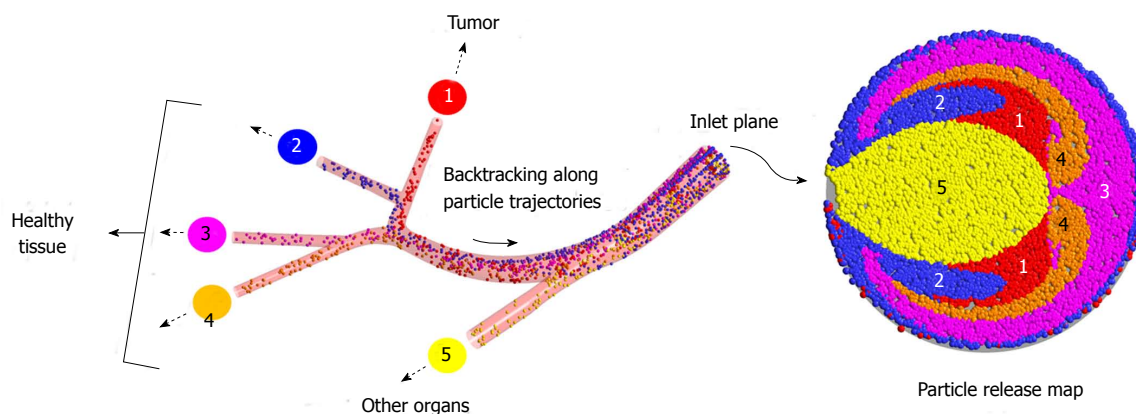


Figure 5 Illustration of the direct tumor-targeting methodology. (Reprinted from Ref.^[115], with permission from Springer).

been achieved by taking advantage of the different pH, temperature, and enzyme levels in and around tumors. Despite these efforts, NPs still face filtration by non-target organs or clearance by the immune system before reaching the tumor^[91,103-105,107].

Magnetic drug targeting

Magnetic drug targeting is one technique for increasing NP accumulation at solid tumors. In this method, drugs are bound to magnetic nanoparticles, and an external magnetic field is applied to attract the particles to a target site. Several studies have demonstrated the feasibility of this approach through computational and animal studies, and a few have demonstrated this targeting in human trials. Recently, studies have focused on obtaining a better understanding of the parameters which affect magnetic targeting success. For example, Kayal *et al.*^[108] experimentally and computationally analyzed the effects of the flow, magnet, nanoparticle properties, and injection site on deposition efficiency. As expected, they found that the efficiency was lower for increased flow rates, lower magnetic field strength, and smaller NPs. These, and other studies, demonstrate that the current applications are limited due to restrictions on the particle size and magnetic field strength. Specifically, this technique is only applicable when the tumor is located near the body surface and the flow rate is small. To overcome this, implant-assisted magnetic drug targeting has been proposed in which a magnetic implant is inserted near the target site to increase the magnetic field gradient in deep tissue^[109]. However, this technique is still not likely to resolve the downstream tumor-targeting problem because it may not be feasible to place these implants as near to the tumor as necessary.

Direct drug-targeting

As an alternative to the current strategies, a novel direct tumor-targeting methodology has been proposed. In this technique, the catheter position (*i.e.*, radial position in the arterial particle-injection plane), infusion speed, and injection timing are precisely controlled so that the injected drug-loaded particles are carried by the blood flow directly to the target site^[110]. While previous work has

demonstrated that the particle infusion speed should be relatively equal to the surrounding blood flow^[111,112], the remaining conditions are determined by computational simulations which mimic the targeted arterial system. Specifically, a vast amount of particles are infused over the selected injection position of the truncated arterial system, and their transport is modeled through the system. By backtracking along the particle trajectories (as indicated in Figure 5), a patient-specific particle release map (PRM) can be generated, which visually links particle injection regions with associated exit branches, some potentially connected to tumors. Such PRMs can then be used to determine radial micro-catheter positions to achieve optimal targeting. For example, in the scenario given in Figure 5, the catheter should be placed in the red zone (zone 1) of the PRM while avoiding the remaining zones. By generating multiple PRMs at subsequent intervals throughout the cardiac cycle, the best injection interval can also be determined^[113].

The computational medical management program:

The Computational Medical Management Program has been proposed to implement this targeting methodology into clinical practice. As illustrated in Figure 6, there are three basic stages in this program: (1) the patient evaluation stage; (2) the computer modeling stage; and (3) the clinical implementation stage.

As in current intra-arterial procedures, the patient evaluation stage includes classification of the tumor and determination of the best treatment route. In the proposed procedure, the patient's geometry and flow conditions are also collected in this stage. In the next stage, computational case studies are run in the truncated geometry to determine the best injection region and interval for targeting as well as the appropriate time to terminate injection. In the final stage, optimal catheter positioning and injection is achieved using the proposed Smart Micro-Catheter (SMC) and Medicine Supply Apparatus (MSA). As in current procedures, success of the treatment is then evaluated.

SMC system for optimal drug-delivery: As introduced in the previous section, a SMC and MSA have been

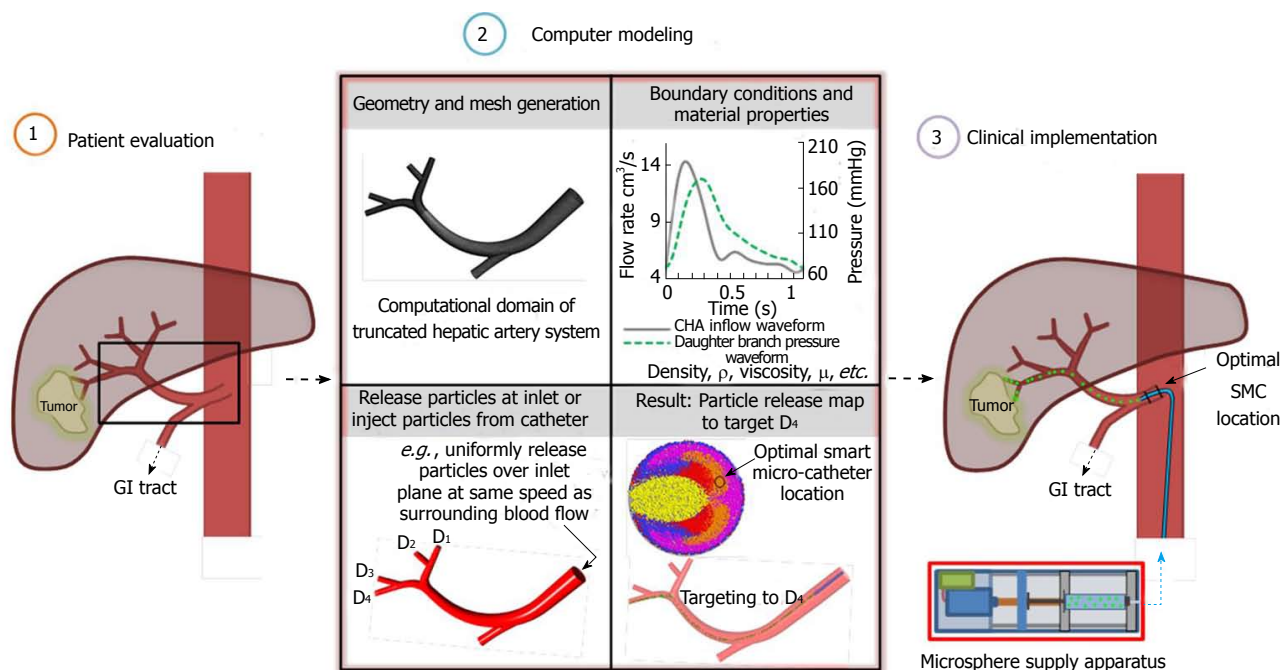


Figure 6 Computational medical management program. SMC: Smart micro-catheter.

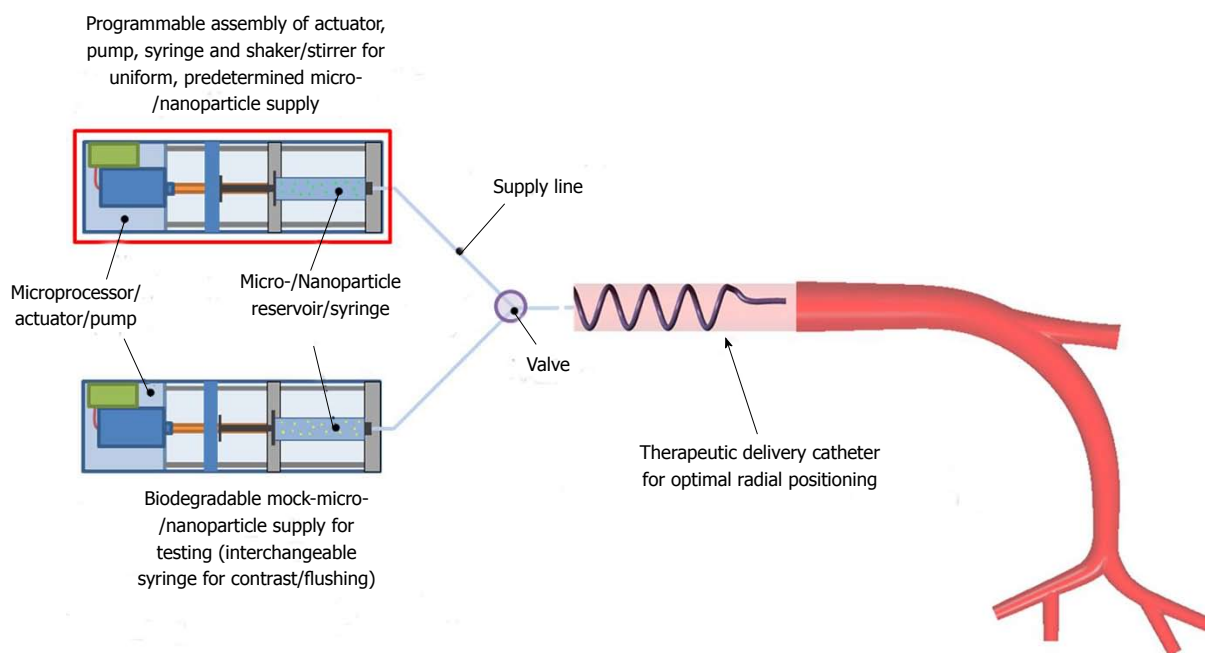


Figure 7 Proposed components of the medicine supply apparatus. (Reprinted from Ref.^[118] with permission from Elsevier).

proposed to achieve direct tumor-targeting. The main objective of the SMC is to provide precise intra-arterial positioning of the particle injection stream, while the main objective of the MSA is to supply the particle stream to the SMC at the appropriate interval and speed for targeting. Figure 7 illustrates sample concepts for each device.

Experimental and computational studies: As an initial validation of this direct tumor-targeting strategy, Richards *et al.*^[114] performed experimental studies in a scaled-up,

rigid hepatic artery system with steady Newtonian-fluid flow. Using the generated particle release map from the corresponding computational simulation, it was demonstrated that specific downstream arteries could be targeted by precisely positioning the particle injection region upstream. While additional computational studies have demonstrated the feasibility of this technique under more realistic conditions such as transient pulsatile flow, a patient-specific geometry, and flexible arterial walls^[111-113,115], future experimental studies will need to verify these findings.

CONCLUSION

In this review article, we compared and discussed different drug-delivery devices and drug-targeting methodologies using pulmonary or intravascular routes, as well as related computational fluid dynamics techniques and applications. Drug-targeting has the potential to greatly enhance drug-delivery efficacy, reduce side effects, and lower treatment cost. However, the vast majority of drug-targeting studies assume that the drug-particles are already at the target site or at least in its direct vicinity.

In this review, drug-delivery methodologies, drug types and drug-delivery devices are discussed with examples in two major application areas: (1) inhaled drug-aerosol delivery into human lung-airways; and (2) intravascular drug-delivery for solid tumor targeting. The major problem addressed is how to deliver efficiently the drug-particles from the entry/infusion point to the target site. Experimental results so far are based on simple laboratory studies and restrictive animal tests. Concerning computational fluid-particle dynamics, further advancements in software and hardware are needed to develop faster, more realistic and accurate computer simulation models.

Pulmonary drug targeting

As mentioned, the selection of drug delivery device and drug aerosol formulation has a critical influence on pulmonary drug-targeting efficiency. To further optimize the pulmonary drug-delivery process and provide more effective therapy, the focus should be on the following aspects: (1) control the aerosol generation process^[119]; (2) control the aerosol deposition patterns in lung airways; and (3) control the aerosol transport after penetrating the air-blood barriers. Specifically, due to the scarcity of air-blood barrier transport of drug aerosols *via* the lung route, it is of interest that to know to what extent drug-aerosols can be absorbed or cleared. That will affect the systemic drug delivery effectiveness, including modulation of solubility in the airway-surface layers and the permeability across the epithelial barrier to improve pulmonary bio-availability of the active pharmaceutical ingredients. It should also control the clearance process to prolong the action of the active pharmaceutical ingredients.

Solid-tumor targeting

It is evident that the micron- or nano-drugs have to be delivered directly from the infusion point to the pre-determined tumor site to guarantee high treatment efficiency, minimal side-effects, and low cost. Such a direct drug delivery equates to optimal tumor targeting. Concerning the promising smart micro-catheter system, *i.e.*, a combined and synchronized SMC and MSA assembly, SMC-device miniaturization and system testing in the lab and clinical environment are ongoing and planned projects.

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Relational coordination and healthcare management in lung cancer

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Abstract

In the current socio-economic scenario characterized by a growing shortage of resources and progressive budget constraints, the need to better coordinate processes in health institutions appears as a relevant aspect to ensure the future sustainability of system. In this sense, Relational Coordination (RC) provides a valuable opportunity for the reconfiguration of clinical guidelines concerning isolated single-level considerations. In this research the RC model has been applied to explain best results in the process of diagnosing and offering clinical treatments for lung cancer. Lung cancer presents the higher rates of tumor's mortality worldwide. Through unstructured and informal interviews with clinicians at both levels (Primary/Specialist Care), a diagnosis of the situation in relation to joint management of lung cancer is provided. Solutions of continuity in terms of coordi-

nation are explained due to the observation of lack of effective knowledge transfer between the two levels. It is this disconnection which justifies the introduction of a modified model of RC for the study and implementation of transfer relations between the knowledge holders, in order to structure consolidated and cooperative evidence-based models that lead to a substantial shortening in the response times with a marked outcomes improvement. To our knowledge, the application of this model to a Public Health problem bringing together both levels of care, hasn't been made till now.

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Key words: Relational Coordination; Lung cancer; Clinical pathway; Dialogic practices; High performance work systems; Healthcare performance

Core tip: Innovative managerial frameworks have to be put into practice when treating severe diseases. Relational Coordination makes possible to enhance inter-level knowledge networks to obtain better outcomes from the perspective of the National Health System and the patients. Through systematic revision, it has been checked that only in the fields of Endocrinology and Psychiatry have these frameworks been applied. This model tries to establish a coordinative solution within the field of Oncology, implementing the Theory of Relational Coordination as a tool to get optimal results in lung cancer.

Romero JAV, Señarís JDL, Heredero CDP, Nuijten M. Relational coordination and healthcare management in lung cancer. *World J Clin Cases* 2014; 2(12): 757-768 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i12/757.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i12.757>

INTRODUCTION

In the current schemes of complex pathologies manage-

ment, collaborative scenarios are required in order to get to an integral coordination of health problems *via* multi-level strategies^[1-3]. This statement acquires higher importance once we consider some concrete disease processes that, due to their increasing incidence and associated costs from both the patient's and the National Health System's (NHS) perspective, constitute core objectives for specific interventions that may reduce time to take accurate decisions headed to resolute, chronify or minimize the pain caused by the disorder. Coordination networks building and optimization of multi-level tacit knowledge transfer, would provide cost containment per patient stay to the NHS and to the social welfare system, contributing this way, to increase Quality Adjusted Life Years decreasing, as well, Disability Adjusted Life Years.

The objective of this study is to apply the Relational Coordination (RC) Model^[4-6] to a determined health problem in order to implement the appropriate treatment trajectories based on continual permutation of actions^[7] that return positive outcomes on the indicators formerly mentioned. Although, as far as we know, this model has proven very good results in hospitals, nothing has been performed using this model to check out the effectiveness of coordination strategies between levels of care, when considering the design of common pathways to treat successfully neoplasia as a serious Public Health concern.

To enforce this objective, lung cancer has been selected as an example of great relevance disorder. This is due to: its really important Disease Load^[8,9], high treatment cost^[10,11], social coverage of disabilities, side-effects on productivity^[12], assistance discontinuity between Primary (PC) and Specialized Care (SC)^[13] and lack of common recognized procedures and pathways between them and within every isolated level.

This study is focused on comparing actual circuits that patients from PC follow until they get to their reference hospital (in this study we consider a high complexity one, Beveridge typology within a National Health System, with its made-to-measure design of Lung Cancer Clinical Pathway), with our proposal of modified, multilevel RC model applied to clinical trajectories that permit assistance continuity.

As a consequence, it is absolutely necessary to build a clinical alert system triggered by proven suspicion of neoplasia. This needs to be in accordance with the next healthcare level (SC) to, under the premise of evidence-based clinical practices, develop quick completion check-lists that enable immediate^[14] transference of patients to the adequate level, using information technologies as preferred communication channel^[15,16].

With respect to inter-level coordination, we can highlight that this is not a clinical routine. As scientific literature points out, this cooperative strategy is put into practice by medical specialties not or discretely related to Oncology, such as Endocrinology or Psychiatry. Actually, we find no evidence of cooperative inter-level strategies being carried out in the field of Oncology^[17]. Difficulties in structuring these kinds of practices lie on the basis of knowledge transfer^[18,19] in organizations characterized by the dominance of its tacit component^[20,21] that rarely

show High-Performance Work Systems (HPWS) practices^[22]. Furthermore, organizational designs for effective and efficient management of oncologic diseases have to be both adaptative and dynamic^[23] to be aligned with the state-of-the-art advances recognized in gold standards.

To this respect, RC appears as a high-traceable bi-directional tool to provide excellence in neoplastic lung disease treatment. The model theorized by Gittell^[5] offers a global coordinative vision of the organizational process, helping to figure out inefficiencies that can be corrected by initializing and implementing cooperative practices and, as a result, proactive organizational designs that tend to rationalize use of resources. Additionally, it contributes to establish optimal relational dimensions for potential efficiencies of scale and scope depending on the attributes of relational and intellectual capital within the organization^[24-27].

A noteworthy aspect of the suitability of the model of choice is supported by the complement that offers for the integration of related scientific approaches within the field of Business Organization, such as Operational Management. This will allow the development of further studies to refine reengineering process *via* supply-chain, thus contributing to the optimization of both the intermediate (surrogate-end-points) and the final results observed in patients (outcomes), and in the income mediated by Risk Adjustment Systems^[28] used in health financing. Thus, if the inefficiencies inherent in the coordination process of the disease are debugged, additional financial returns (based on capitation criteria related to the number of processes) could be achieved (even from the health organization's perspective with regard to cost-efficiency). Another feature that makes Gittell's^[5] model ideal for our purpose, is that it previously develops a series of tables analyzing HPWS practices, correlating its absence or presence with RC and, consequently, with final outcomes. Thus, the same model before being implemented, allows a diagnosis of the situation in terms of the practices mentioned. Once conclusions resulting from field work have been extracted, the implementation of the model can lead to the development of the absent figures, resulting in a multidirectional dynamic feedback identifiable with the continuous improvement cycle^[29].

Moreover, and in a methodological approach, given the intangible nature of the concepts that promote operational developments in the organization as well as their interaction and mutual influence, the model enables the application of multivariate analysis techniques focused on structural equations. This has the potential to provide greater rigor and validity when checking the assumptions in a context of real research.

In addition, *via* RC valuable information it would be possible to extend the network (regarding the possibility of generating "Value Networks"^[30] in the process) to other stakeholders such as pharmaceuticals and health technology companies, and even to managerial superstructures committed on public health concerns.

Incorporating the principles of Business Organization to the clinical setting is absolutely essential to promote

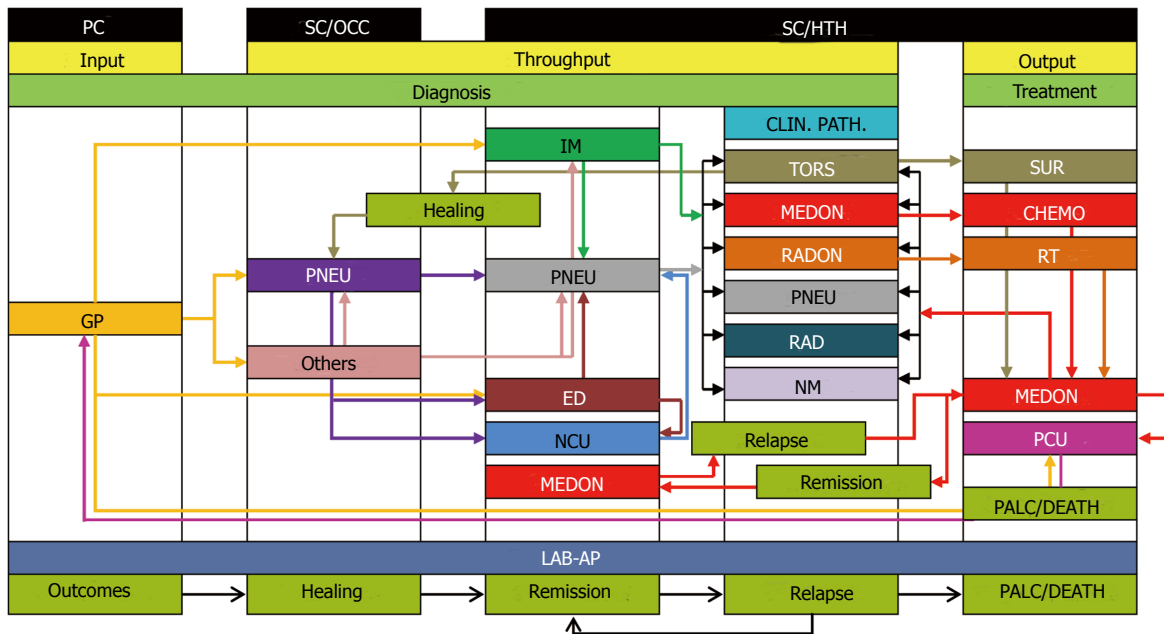


Figure 1 Inter-level process map. Source: Authors' own elaboration. PC: Primary Care; SC/OCC: Specialist Care/Outpatient Care Center; SC/HTH: Specialist Care/High-Tech Hospital; GP: General Practitioner; PNEU: Pneumology; IM: Internal Medicine; ED: Emergency Department; NCU: Neoplasia Consulting Unit; MEDON: Medical Oncology; TORS: Thoracic Surgery; RADON: Radiation Oncology; RAD: Radiology; NM: Nuclear Medicine; SUR: Surgery; CHEMO: Chemotherapy; RT: Radiotherapy; PCU: Palliative Care Unit; LAB-AP: Clinical Laboratory and Anatomical Pathology Services; PALC/DEATH: Palliative Care/Death.

the reduction of redundant, iterative processes in traditionally knowledge intensive organizations. These have ignored their role as business managers of a huge amount of resources, in interest of a pretended clinical excellence oriented to complex phenomenology and high media impact in terms of prestige, influence and fund raising, but with little residual value in the generation of efficiencies of scale and scope, given the peculiarities involved in tertiary healthcare.

Given the above, the model postulated by Gittel^[4,5] having been tested previously^[6] in these types of organizations, is an excellent reference for the detailed study of the relationships involved in designing healthcare procedures between the different levels. It provides the basic tools for understanding and constructing a single treatment line that does not register any undesirable delays attributable to gaps in knowledge and other factors, including relational. The model also facilitates the continuous review of the adequacy of the approaches proposed and its comparison with reference standards in relation to screening, extension study, staging, treatment, monitoring and rehabilitation of lung neoplasia.

INTER-LEVEL PROCESS MAP AND LUNG CANCER CLINICAL PATHWAY IN A HIGH-TECH HOSPITAL OF A NATIONAL HEALTH SYSTEM

Before accessing the clinical pathway itself, there are many routes to be made by patients in their journey between the two levels of care. This route identifies itself

with the diagnosis and treatment of the disease, and represents the transition between access to primary and secondary healthcare (Figure 1). For operational reasons, it excludes access to tertiary level (rehabilitation).

To carry out the process map, unstructured interviews during the months of March, April and May 2012 have been developed with Pneumologists and General Practitioners (GP), as key agents in the user's address to the specific resources for diagnosis and treatment.

The patient's contact with the system starts at the level of PC in the Health Center, where the person requests for consultation to GP due to a series of signs and/or symptoms which may be more or less related to tumors. At this point, a series of basic tests as conventional radiology and blood analytics are required. It is here, where they may be starting to produce the first delayed diagnosis attributable to the transfer of knowledge not based on evidence, since these blood tests not always include non-specific tumor markers, but indicators of malignancy. Furthermore, in many cases, treatment is initiated based on empirical diagnosis of pneumonia, since the neoplastic process can be masked or not being clearly visible by conventional radiology.

From this first GP visit, a second one is requested for reevaluation of improvement and/or worsening of the patient. Also in this second visit, imaging and laboratory results are delivered. This time period can vary from one week to fifteen days (period in which the empirical treatment of antibiotic action produces noticeable changes in the patient's condition and laboratory data). At the persistence of symptoms or onset of signs clearly suggestive of clinical suspicion, inter-consultation part is issued to

the specialty of Pneumology (SC) based at an Outpatient Care Center (OCC). This will lead to an additional delay, although it may be attenuated in case of emergency.

While the above, it should be noted that this path is followed if the clinical manifestations clearly compromise the patient's respiratory status. However, there are not always reliable evidences of pathology as they may be semi-hidden or produce "*a priori*" events unrelated to the disease. This can lead to other specialties not specifically connected with the process, due to the absence of effective knowledge transfer between levels.

Consider, for example, the case in which the disease is indirectly manifested through referred pain in locations as knee or scapula, with inconclusive imaging tests and non-specific tumor markers requested either. In this case, when a person with referred pain consults GP and also has a history of degenerative joint disease, the first inclination is based on the therapeutic application of trauma imaging at the involved location. If this do not show alterations, to an extension diagnosis, the patient maybe referred to other specialties, *via* interconsultation, at the level of OCC, as may be Rheumatology or Traumatology (again, we would have another delay in the issuance of a firm diagnosis). To this must be added, the additional delay in the appointment date for new consultations, delays in the completion of tests that are to be applied in these services and the appointment date for review. Note, that the pain reported by the patient could be produced as a result of neurological compression due to tumor growth.

Once the presence of specific pathology related to other specialties different to Oncology is ruled out, and for a possible occurrence of respiratory and/or systemic signs, the patient may be referred back to the GP (who will likely refer the patient to another specialist in the short-term, as for example an Internal Medicine practitioner at the hospital level) or Pneumologist at OCC level.

It is vitally important to consider that these successive accumulated delays entail delayed diagnosis and may incur progression of the disease from an early stage potentially curable, to an irreversible stage where only palliative measures can be adopted.

Special mention deserves the situation in which the patient attends a first GP consultation when the disease is in an advanced stage. In this case, the GP may take two decisions: one would be immediate referral to the reference hospital emergency department. The other one would be derivation, equally immediate and avoiding waiting list, to Pneumology at OCC.

The tours in both situations are as follow. In the case of referring the patient to the high-tech hospital emergency department considered for the study, the user would be admitted in the lower course of time. Alternatively, the physician at SC may order scheduled or urgent admission according to his criteria, and even *via* emergency if the clinic is accused and limiting.

Returning to the circuit in which the disease is not clearly delimited, and placing the patient back to Pneumology at OCC, application of tumor markers and more

specific diagnostic imaging tests with their corresponding waiting times will be issued. This will finally lead to a first diagnosis of neoplastic disease, although in many cases progress is relentless, having passed the tipping point between curability and elongation of survival.

Once the diagnosis has been confirmed (after issuance of radiological and pathological opinion), the Pneumologist at OCC (based on clinical signs) will apply for patient admission at the Respiratory Unit at the reference hospital or at the Neoplasia consulting Unit (staffed by Pneumologists), also located at the hospital but belonging to the outpatient section (OCC/HTH), who predictably will speed his admission to the Respiratory Unit.

It has to be taken into consideration that, as reflected in Figure 1, in cases of nonspecific symptoms the GP or the specialist physician from other disciplines different to Pneumology, can request patient admission at an Internal Medicine Unit. Once there, and after confirming diagnosis, the patient will be placed in charge of the service consulted (Pneumology) or included directly in the clinical pathway in order to be admitted to the Respiratory Unit.

As it has been explained, to reach this clinical decision, the circuits followed by patients are often redundant and inefficient incurring incremental health expenditure and associated loss of productivity, with the aggravating factor of disease progression, which dramatically affects the effective resolution of the process due to a probability increasing of metastatic dissemination. The next step is the inclusion of the patient in the clinical pathway itself.

The Clinical Pathway Commission is a multidisciplinary board delegated by the Chief Medical Officer of the hospital, whose mission is to take collective decisions about the individualized treatment for each patient given the specific types of lung neoplasia presented. It consists of various clinical services such as Thoracic Surgery, Oncology, Radiation Oncology, Pneumology, Radiology and Nuclear Medicine, and meets in session once a week to discern treatment strategies addressing cases that present criteria for inclusion.

Previously, and once the patient has already been admitted in hospital, sequential examinations to limit the spread of the disease and rule out metastasis are conducted.

Once all results are available, which will generate a new lengthening of waiting times due to internal procedures, they are presented in clinical session to elucidate what the best combination of medical and surgical options is. In this regard, it is noteworthy to point out several strategies that, in turn, generate new decisional and time-restricted flows around the patient.

First, and once the kind of broncho-pulmonary neoplastic lesion is typified, in case that the option of surgery is the one chosen, the patient is transferred to Thoracic Surgery (which acts only in relation to the surgical process) being the customer the Respiratory Unit. Upon resolution of this action, the patient can follow two routes

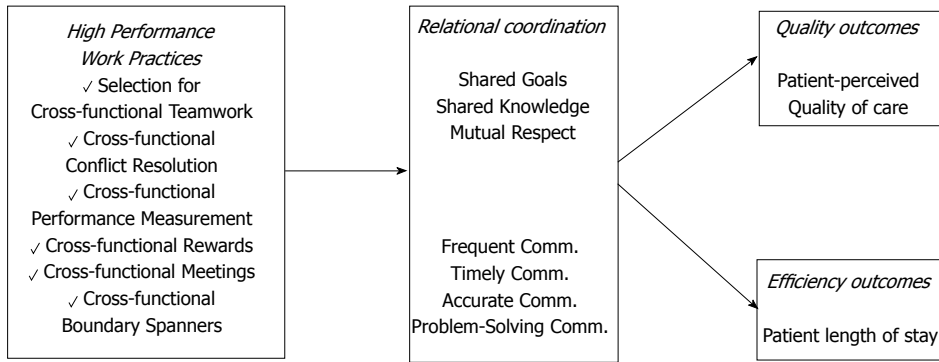


Figure 2 Relational coordination model. Gittell^[4] (2009).

depending on the characteristics of the disease. If the lesion was curable with just surgery, if it is in a nearly stage, the subject is again brought back to the customer service where periodic reassessments continue on an OCC basis. If clinical pathway was structured in a combined plan of several therapeutic strategies, the patient will be assigned to Medical Oncology and submitted to treatment schemes consisting of chemotherapy and/or radiotherapy (if appropriate), acting as coadjutor service, Radiation Oncology.

Following this, we have to clarify the types and purposes of cancer treatments and their relationship with Thoracic Surgery, as well as with the alternative circuits of patients arising as a result. These treatments can be neoadjuvant or adjuvant. If a neoadjuvant treatment is preferred, the patient will be referred to Medical Oncology from Pneumology, where after receiving the appropriate treatments, will be referred to Thoracic Surgery, and once its performance has concluded, he will be returned to Medical Oncology where the patient will ultimately be assigned. If the *adjuvant* treatment option was considered, the bypass sequence would start from Pneumology to Thoracic Surgery and from the latter to Oncology, where, likewise, the patient would stay finally allocated.

In the case (very common) that Radiation Oncology intervenes, the customer service would be Medical Oncology, and the role of Radiotherapy would be the imposition of costs derived from treatment processes to the original customer.

While all of the above, it is another distinct possibility in case the lesion is unresectable. In this situation, the Commission may choose the administration of chemotherapy and/or radiotherapy to ensure the stabilization of the process. In this sense, the patient is finally moved to Medical Oncology where clinical decisions and treatments are provided, appearing again the interactions described above for the Radiation Oncology department.

After running all the steps above, the circuit would continue in different ways to transfer responsibilities between services and even between levels, depending on the clinical outcome. Thus, we would obtain the following sequences: (1) healing: The patient would return to Pneumology at the OCC level for regular checkups; (2) remission: If partial or complete remission, the patient

would be discharged and would attend appointed check-ups at Oncology Day Hospital (OCC/HTH); (3) relapse: In this case, the patient re-enters in charge of Medical Oncology, without his case again be subjected to clinical pathway for application of second-and subsequent lines; and (4) palliative Care/Death: For unsolvable progression of the disease, the patient is transferred from Medical Oncology to Palliative Care Unit (PCU), where treatment is merely symptomatic. Nevertheless, depending on the patient's general condition and in respect of his decision making capacity (if this was preserved or had been previously stated), the patient can be treated at home through a collaborative partnership between PCU (SC) and GP.

Restructuring of patient inter-and intra-level flows is absolutely needed. It requires corporate collaborative systems providing knowledge to one medical act. Therefore, the role of the RC model acquires great relevance as it acts as mediator and promotor of joint strategies aimed at improving results, from both, the patient's and the NHS' viewpoint.

RELATIONAL COORDINATION MODEL

Based on previous studies grounded in mutual adjustment^[31-35] and coordination approaches based on relationships^[14,36-40] in corporate environments of high/low interdependence/uncertainty, Gittell develops her model (Figure 2) as a contribution to the study of relational dynamics.

In this sense, Gittell^[5] defines her model as a mutually reinforcing process of interaction between communication and relationships carried out for the purpose of task integration. She also states that her theory differs from others due to the proposition of three specific relational dimensions that are necessary for effective coordination.

While many other recent theories emphasize the importance of shared knowledge, RC argues that while this is necessary, is not sufficient. Accordingly, to achieve effective coordination, the members must also be connected through the possession of shared goals and mutual respect.

As mentioned in the previous section, role-based coordination has an advantage over coordination based on

personal ties. While the former may require more investment for being implemented, role-based coordination fosters a role exchange that encourages corporate flexibility to adapt to changing environments within a high uncertainty and interdependence frame over time.

The model is structured around two types of dimensions: Communication and Relationship.

Within the dimensions of communication, we find:

(1) frequent communication: Frequent communication helps to establish relationships among roles through the closeness generated as a result of repeated interaction^[5]; (2) timely communication: Delays in communication may have negative implications for organizational performance. Hence, the importance of timely communication, and at the right time, for successful development of organizational tasks^[41]; (3) accurate communication: Accurate communication, regarding the content of relevant information, plays a critical role in the effectiveness of group tasks performance^[42]; and (4) problem-Solving communication: Effective coordination requires that those involved in the task, engage in communication to solve the problems that appear in a group performance characterized by high interdependence (rather than blame others involved or evading the responsibilities). This can lead to negative consequences that singularly affect performance^[29].

Within relational dimensions in Gittell's model we find: (1) shared goals: This aspect plays a key role on the coordination of highly interdependent tasks^[43,44]. Using a set of shared goals regarding the work process, involved individuals develop ties that allow them to reach conclusions that are compatible with the different ways of thinking and acting as new information is available^[5]; and (2) shared knowledge: While Dougherty^[45] points out that communication among those involved in the various tasks that constitute a process is not always effective because of different social backgrounds, training and/or experience, Gittell^[5] states that when members know how their tasks are related to other members within the same process, it creates a dynamic in which everyone knows the impact that each change will reflect on each task and each role; and (3) mutual respect: Respect for the competence of others involved in the process, provides a powerful tie that will be implemented in a comprehensive way across the whole process generating, as a result, an effective coordination^[5].

Through this design, it follows that the RC model turns into a model of intensive coordination in communication and relationships, particularly useful to achieve higher levels of performance under high levels of interdependence among tasks, uncertainty and time constraints. So, it is an example of process improvement that allows a work group department or organization, raise their production possibility frontier to more favorable positions while achieving higher efficiency and quality^[5].

It is, therefore, to achieve work processes improvement through improving the quality of labor relations

among the actors that play different roles in these processes, thus leading to a higher quality of communication. Through this procedure, it is intended to reduce errors delays and redundancies observable among tasks interdependences within the critical organizational processes.

Model measurement is done through validated surveys to participants in a given process, on the activities of communication and relationships with others involved in the same process, with whom relations of interdependence are kept for achieving the same common objective. Related to the above, the first step would be the selection of a work process that serves a population of interest. Then, the roles or functional groups embroiled in the development of the focal process must be identified. The third step would consist on identifying which of those groups could be accessed by the researcher in order to develop matrices (symmetric/asymmetric) of RC links.

Once the previous steps have been run, likert-type survey would be delivered to the participants consisting of seven questions: four on communication and three concerning relations between different process roles. In order to reduce possible bias attributable to socially desirable responses, RC's survey asks participants about behaviors of other roles, except for organizational behavior feature represented by the frequency of communication. In addition, questions are referenced to habitual patterns of behavior, rather than be directed towards identifying specific events, that are part of the current patterns in the time of evaluation. Thus, avoiding erroneous response patterns based on retrospective response biases.

The characteristics of the model applied to the investigation of coordination practices in the management of interdependent processes, allow multi-functionality in analytical and exploratory orientation, presenting four complementary and inclusive perspectives that give great versatility in building research designs. Thus, the model can be used with the next main purposes: (1) analysis of the effect of RC on organizational performance: Determination of the impact of the model on improving the quality and efficiency of a given process characterized by high levels of interdependence, uncertainty and time constraints; (2) analysis of predictive factors of RC: Determination of the effects of organizational practices to use on the model; (3) analysis of the mediating effect of RC: Determination of the influence of the model on existing organizational practices and their impact on results; and (4) analysis of the moderating effect on RC: Determination of the influence of a/some given factor/s on the effect of the model on organizational performance measurement.

Next, we describe the proposed model for this paper based on RC modified at its core by adding two features of organizational behavior that are interrelated. Also, we introduce two moderating factors to analyze the mediating effect of it and its impact on organizational performance, taking as a critical process multilevel management of health institutions in relation to lung cancer.

To increase consistency and operational development in the future, a comparison with institutional practices currently applied (particularly single level clinical pathways) is made.

APPLICATION OF A MODIFIED, DOUBLE-MODERATED MODEL OF RELATIONAL COORDINATION IN PERFORMANCE MEASUREMENT OF LUNG CANCER MULTI-LEVEL TREATMENT TRAJECTORY VS ISOLATED, SINGLE-LEVEL CLINICAL PATHWAYS

As it has been previously described, the main objective of this paper is the proposal of a modified, double-moderated model of RC (Figure 3), to investigate the degree of development of the theory proposed by Gittel^[5] and the impact of its implementation on HPWS practices and outcomes obtained by the institution. In reference to the above, the greatest achievement of this study is supported by theoretically increased times of disease-free survival (DFS) as a consequence of optimal-efficient redesign of coordination strategies between levels involved in diagnosis and treatment. In addition, the potential gains highlighted would imply healthcare costs containment that would return positive savings on other important health problems, being possible to achieve true clinically-oriented corporate strategies.

First, and as a previous step for defining the implications of the proposed model on performance, we need to identify HPWS practices currently observable at health organization level (Table 1) and at the level of diagnosis and treatment of broncho-pulmonary carcinoma (Table 2) for both care providers (PC/SC).

As seen on Table 1, HPWS practices in the health care organization have a wide variability and segmentation that result in continuity of care disconnection. Furthermore, the absence of inter-level operational relations in critical aspects emphasizes the fact pointed out, generating a discontinuity of the care process that could lead to very significant increases in transaction costs, as well as duplication of health expenditure and intangible consumption.

This situation is much more serious when looking at data provided in Figure 2, referred to the specific coordination process of the pathology. In this case, it highlights the virtual absence of joint strategies for the management of the disease. In light of this information, the existence of redundant and iterative processes (that create delays in the effective application of therapeutic measures associated with consequent costs due to disabilities and/or productivity losses), gets verified.

The comparison of data shown in Tables 1 and 2 has been made through unstructured interviews with senior doctors at a health center, which has as reference hospital the one mentioned throughout this work, and that is very

representative due to the volume of population served (approximately 22000 health cards).

It justifies the introduction of a modified model of RC for the study and implementation of transfer relations among knowledge holders, in order to structure consolidated, evidence-based cooperative models that would lead to a substantial shortening of response times with a marked improvement in outcomes.

The sequential development of our model is based on the examination of the organizational behavior considered by Gittel, using the methodology annexed to the theoretical approach^[5]. Then, it will be complemented by a combination of studies of other authors in the field of management, which postulate the study of trust relationships^[21,46] and dialogic practices^[14] as effective methods of organizational coordination.

These are especially relevant for high-uncertainty processes requiring quick and adaptive replies.

Related to the above, trust is a predictor and a consequence of interpersonal relationships^[21]. In this sense, a higher degree of trust acts as a stimulating factor for communication accessibility, promotes greater effort from those involved in a task and reduces conflict in work teams, fostering better results in performance^[47,48]. It could be inferred that it acts as an enhancer of RC.

Moreover, dialogic practices tested in high-uncertainty hospital services^[14] appear as coordinative solutions (from a point of view focused on process trajectories^[7]), by which cooperative guidelines are structured (Figure 4). This fact is particularly important, given the high variability inherent to the process of diagnosis and treatment, and the consequences of a not entirely optimal assembly of tasks committed with the successful resolution within a framework of time-coordinated action.

Related to approaches focused on trajectories^[7], these are described as goal achievement oriented sequences of action that emphasize contingencies and interactions among those involved in, differing from routines in that the latter merely emphasizes a sequence of steps that can't be extrapolated to work situations characterized by novelty, unpredictability and changing environments in relation to tasks, actors and resources^[14].

So, it is about measuring the degree of trust that the different professionals at both levels have in dialogic practices, following the methodology used by Dietz and Hartog^[46] (Figure 5). This evaluation methodology would be applied to the PC level on the SC level and *vice-versa*.

In a structured way, it would be done a pre-test evaluation of the RC status (for a given level of trust in dialogic practices) at an early stage at both levels, applied to the target health problem. These data would be correlated with the evidence about HPWS developments.

Subsequently, a series of actions would be designed to strengthen relational ties between the two levels. For example, formal assistance on regular basis of one PC-GP to the meetings of the Clinical Pathway Commission (SC), with the aim of promoting the practices mentioned as of excellence, can be suggested. As a consequence,

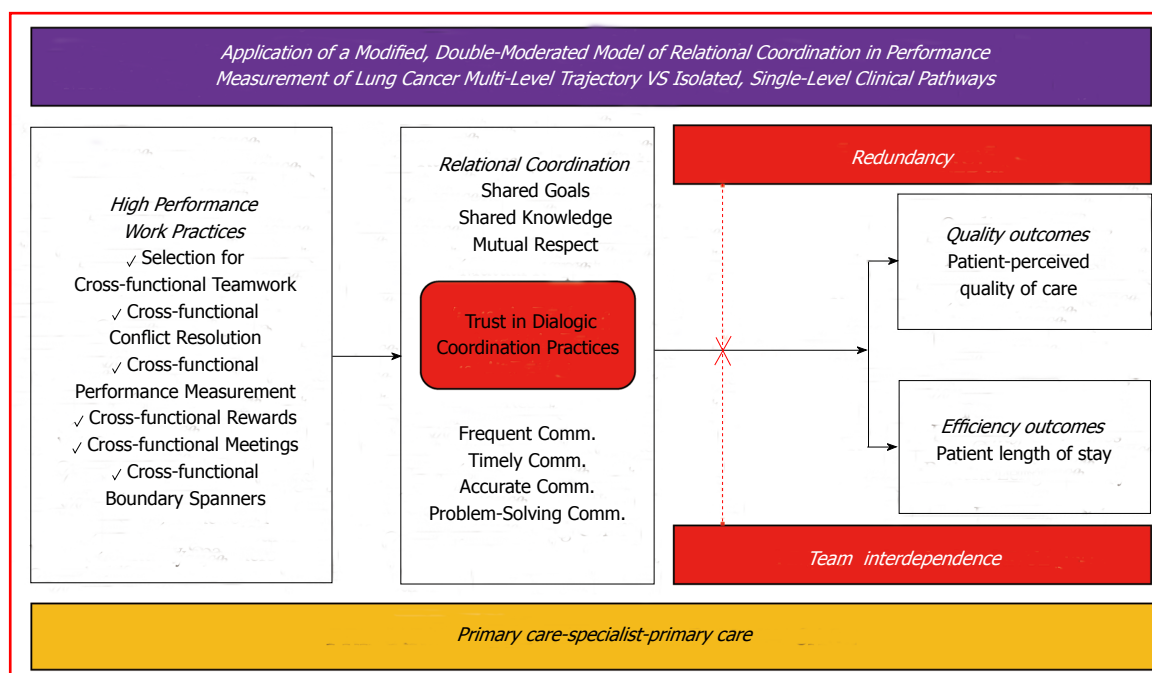


Figure 3 Modified, double-moderated model of relational coordination. Source: Authors' own elaboration.

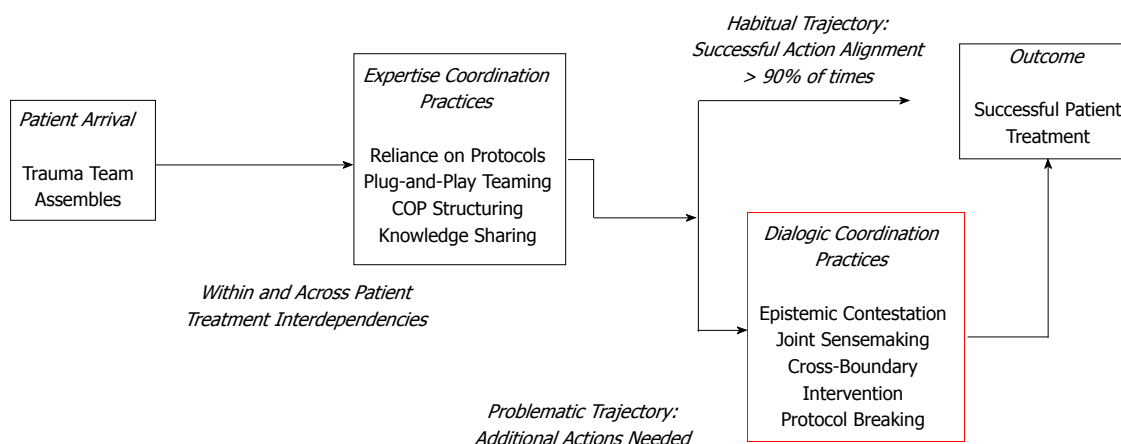


Figure 4 Coordination-focused model of trauma patient treatment. Faraj *et al.*^[14] (2006).

the development of inter-level HPWS practices would be promoted, development that could materialize in creating joint Clinical Practice Guidelines which would contribute to early detection of lung cancer.

Furthermore, to streamline the circuit followed by the patient, direct referring to specific knowledge resources located on SC is intended, always ensuring the traceability of the patient's health status *via* widespread use of shared databases.

In this sense, it is about promoting the representativeness of the Continuity of Care Coordinator (see Table 1) in bidirectional management of patients affected.

Secondly, the interdependence of tasks generated under the prism pointed by Van de Ven *et al.*^[35] would be considered in terms of work flows at the team level. Participating GP's would be enrolled in a training program of High-Fidelity Clinical Simulation following a method-

ology of Objective Structured Clinical Examination^[49-51] that would be taught by specialist physicians specifically trained in this type of methodology. Once these actions have been carried out, a new post-test measure of the degree of trust in RC and dialogic practices would be done at the same time that HPWS practices are re-evaluated.

From this point on, it is necessary to evaluate (retrospectively/prospectively) the time passed since the first contact with the system takes place until the patient receives the first therapeutic action for treatment, prior to and after the implementation of measures aimed at encouraging the development of RC and dialogic practices. Also, as a control measure that would reinforce the theoretical assumptions (raised by some a priori confirmatory hypotheses for the model proposed vs. single level clinical pathways), TNM staging category (at the time of diagnosis confirmation) and estimation of DFS would be

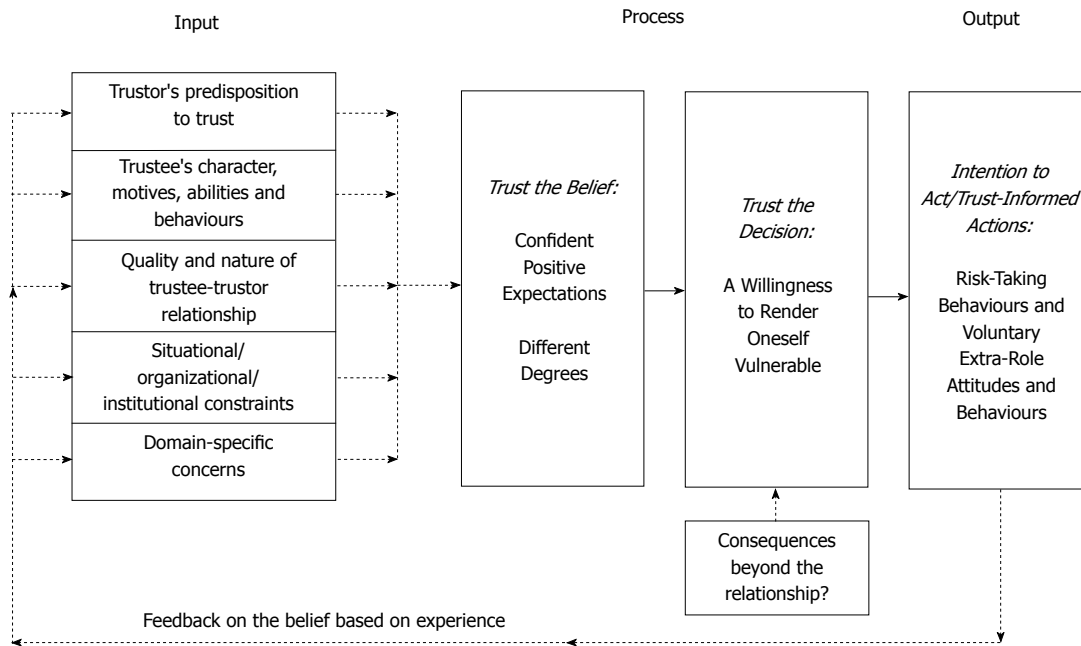


Figure 5 Depiction of the trust process. Dietz *et al*^[46] 2006.

Table 1 High-Performance Work Systems Practices-Health Care Organization

HPWS Practices	Level		
	PC	SC	Inter-level
Selection for Cross-Functional Teamwork	Health Plans	Clinical Pathway Commission Hospital Commissions	Non-existent
Cross-Functional Conflict Resolution	Managing Director at Primary Care Center	Hospital Board of Managers	Deputy Medical Care Continuity (Since October 2010)
Cross-Functional Performance Measurement	Primary Care Center	Clinical Units	Non-existent
Cross-Functional Rewards	Professional Categories at Primary Care Center (Pre-established at 100% Objective Compliance)	Professional Categories at Clinical Units (Pre-established at 100% Objective Compliance)	Non-existent
Cross-Functional Meetings	Primary Care Center	Hospital Board of Managers, Hospital Technical Commissions, Medical Services Commissions	Health Area Managing Board
Cross-Functional Boundary Spanners	Managing Director at Primary Care Center	Hospital General Manager	Deputy Medical Care Continuity (Since October 2010)

Source: Authors' own elaboration. HPWS: High-Performance Work Systems; PC: Primary Care; SC: Specialized Care.

Table 2 High-Performance Work Systems Practices-Lung Cancer Diagnosis and Treatment

HPWS Practices	Level		
	PC	SC	Inter-level
Selection for Cross-Functional Teamwork	Non-existent	Clinical Pathway Commission	Only at Palliative Care Level
Cross-Functional Conflict Resolution	Non-existent	Clinical Pathway Commission/Chief Medical Officer	Non-existent
Cross-Functional Performance Measurement	Non-existent	Clinical Pathway Commission (No. of patients included in the clinical pathway)	Non-existent
Cross-Functional Rewards	Non-existent	Non-existent	Non-existent
Cross-Functional Meetings	Non-existent	Clinical Pathway Commission	Non-existent
Cross-Functional Boundary Spanners	Non-existent	Clinical Pathway Commission	Non-existent

Source: Authors' own elaboration. HPWS: High-Performance Work Systems; PC: Primary Care; SC: Specialized Care.

done. In closing, costs related to hospital stays savings (as a result of early detection) would be calculated.

The proposed model goes from an exploratory qualitative design to (after an inductive process) an explana-

tory type, which generates synergies in the use of scarce resources through coordinative economies of scope.

CONCLUSION

In the current socio-economic scenario characterized by a growing shortage of resources and progressive budget constraints, coordinated process management in health institutions appears as a relevant need to ensure the sustainability of the medium/long term.

The fact that a health system is strategic for a country (particularly if this system is public and universal), together with the sheer volume of resources associated with healthcare provision, makes mandatory to impose a rational logic in the clinical management of public health concerns. This logic has to be even more intense, when it comes to diseases on the rise and rate of disability and/or mortality as the one we are considering in this paper.

In this sense, RC provides a valuable opportunity for the reconfiguration of clinical guidelines concerning isolated single-level considerations, to turn them into cooperative inter-level ones.

Through unstructured and informal interviews with clinicians at both levels (Primary/Specialist Care), diagnosis of the situation in relation to joint management of lung cancer is made, noting that solutions of continuity (in terms of coordination) are observed due to the lack of effective knowledge transfer between the two levels as a result of RC practices absence^[52].

Is in this way, where it is theoretically inferred that delays secondary to ineffective coordination would be attenuated if launching and implementation of cooperative schemes (including relational trust in dialogic practices) had been run. In turn, these strategies would result in the generation of “*ad hoc*” Clinical Practice Guidelines (equivalent to HPWS in other corporate environments), which would strengthen the mission of RC. In this regard, it is worth highlighting the flexibility provided by Gittel’s theory^[5]. It allows adaptation and modeling of its precepts to the organizational conditions of actual practice, providing a specific management architecture for knowledge intensive organizations that remains under heavy pressure on casuistic, uncertainty and technological changes.

The model proposed in this research, advocates a regularization and training of new skills and relational attitudes arising from the collaboration between the two levels of care involved. This consideration is particularly relevant if we return to the defining characteristics of health systems in which advances in the state of the art occur constantly and must be properly transferred.

The operational implications of the suggested theoretical alternative pursue a dual purpose on results. So on the one hand, facilitating diagnosis in early stages of broncho-pulmonary carcinoma, allows more cost-efficient measures, which will return a decrease in rates of morbidity and mortality and a significant reduction in opportunity costs from the patient’s point of view. This would be translated into tangible gains in DFS and the

appearance of negative costs obtained from savings in productivity loss avoiding and containment of hospital stays per process.

On the other hand, the second approach is characterized by the impact on health spending of Medical Care Variation. Because physicians are resource allocators, this scheme of coordination is intended to reduce duplication in diagnostic tests and clinical times. Here appears again the concept of opportunity cost, but in this case, from the point of view of the NHS. If the process is clearly predefined, there would be a bidirectional action sequence that would lead up to a regulated and bottom-up asset allocation from an optimum-efficiency criteria based on clinical excellence and non-repetitive processes. The savings generated by this approach can, in turn, be reinvested in the process itself or moved to other singular processes of great clinical significance.

The research model is, therefore, to efficiently redirect health expenditure incurred by the disease through proactive inter-level and inter-professional coordinative solutions, achieving, this way, improvements in quality of life and survival of patients affected.

It should be noted that the health organization is not to stay longer away from the improvement proposals dropped from the corporate world, because it is in itself a great company if we pay attention to budget, employability and management processes underlying their daily operation.

In this sense, this document is made taking into account the feasibility and methodological orientations arising from the healthcare business world and academia, providing a new strategic vision of the organization of clinical processes in high complexity health corporations.

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New targeted therapies for breast cancer: A focus on tumor microenvironmental signals and chemoresistant breast cancers

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recently entered clinical trials in mono- and combination therapy in metastatic and chemo-resistant breast cancers. Anticancer epigenetic drugs, mainly histone deacetylase inhibitors and DNA methyltransferase inhibitors, also entered clinical trials. Because of the complexity and heterogeneity of breast cancer, the future in therapy lies in the application of individualized tailored regimens. Emerging therapeutic targets and the implications for personalized-based therapy development in breast cancer are herein discussed.

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Key words: Breast cancer; Microenvironment; Signaling molecule; Targeted therapy; Chemoresistance

Core tip: Emerging therapeutic targets may overcome chemoresistance in breast cancer.

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Abstract

Breast cancer is the most frequent female malignancy worldwide. Current strategies in breast cancer therapy, including classical chemotherapy, hormone therapy, and targeted therapies, are usually associated with chemoresistance and serious adverse effects. Advances in our understanding of changes affecting the interactome in advanced and chemoresistant breast tumors have provided novel therapeutic targets, including, cyclin dependent kinases, mammalian target of rapamycin, Notch, Wnt and Shh. Inhibitors of these molecules

INTRODUCTION

The incidence of breast cancer, the most common cancer in women and the second cause of cancer death in women worldwide^[1,2], is currently growing^[3,4]. Cancers are diseases characterized by aberrant microenvironment and intrinsic signaling causing a continuous proliferation of affected cells ("cancer cells"). Clinical features and

prognosis of cancers vary tremendously according to the tissue and organs they originate from and affect. Breast cancer may start in milk ducts, and can be invasive [invasive ductal carcinoma (IDC)] or not (ductal carcinoma *in situ*). IDC would represent up to 80% of cases^[5,6]. Breast cancer may also start in the lobules, with invasive features (invasive lobular carcinoma) or not (lobular carcinoma *in situ*). In metastatic breast cancer malignant cells originating from breast primary tumors invade other tissues and organs of the body, resulting in a systemic disease. As disease early detection is associated with better prognosis, screening campaigns involving healthy female subjects are performed worldwide. Notably, mammography, which requires the use of low-dose X-rays to capture images inside the breast, is the current gold standard screening for detection of breast cancer asymptomatic cases^[7,8]. However, although the technique requires X-rays, the benefits of the earlier detection of breast cancer outweigh the risk of radiation exposure, which can be associated with the development of breast cancer in previously healthy women is present^[9,10]. New approaches for early detection have been proposed, and may also contribute to reducing breast cancer mortality (for review see^[11,12]).

Three major therapeutic approaches are used today to treat or control breast cancer: surgical removal of primary tumors, irradiation of cancer cells to stop their growth, and anticancer drugs, which kill cancer cells or inhibit their proliferation. Notably, oncoplastic surgery, a technique combining classical lumpectomy (or partial mastectomy) and plastic surgery techniques have revolutionized breast-conserving surgery for removal of lumps and malignant masses. However, surgery or radiotherapy still requires chemotherapy to eradicate remaining malignant cells and impede relapses. Anticancer drugs are based on three therapeutic approaches: (1) the classical chemotherapy, where cancer cell proliferation is stopped by the indiscriminate targeting of rapid cell divisions in the body; (2) hormone therapy, devised to stop cancer cell growth by targeting the receptors and downstream signaling molecules of hormones pivotal for the proliferation of these cells; and (3) the emerging and promising targeted therapy, where signaling pathways deregulated in primary breast tumors are specifically targeted. Breast cancer treatment is still challenging, as drugs in use are expensive, have serious undesired effects^[13-15], and drug resistance is common, particularly in metastatic cases^[16,17], underlying the need for new targeted therapies. Interestingly, recent advances in the understanding of breast cancer biology have highlighted the tumor microenvironment as a major player in breast carcinogenesis and have provided new avenues for targeted therapy.

The present review summarizes and discusses the current understanding of changes affecting breast microenvironment during breast tumorigenesis, with a particular emphasis on signaling pathways currently targeted for therapy and emerging therapeutic targets. Personalized-based targeting implementation is also discussed.

TUMOR MICROENVIRONMENT IS PIVOTAL FROM BREAST CANCER INITIATION TO METASTASIS

Numerous stromal cell types are found in the extracellular matrix of the breast stroma, including endothelial cells, fibroblasts, adipocytes, and resident immune cells^[18]. In addition to these cell types, cancer-affected microenvironment contains malignant cells termed as cancer-associated fibroblasts (CAFs), which are the most numerous cell type, and infiltrating macrophages termed as tumor-associated macrophages (TAMs).

Cancer-associated fibroblasts

CAFs were reported to play key roles in malignant cell proliferation and tumor maintenance^[18,19]. An *in vivo* study involving xenograft of MDA-MB-231 breast cells in SCID mice revealed that CAFs induce p53-dependent antimitogenic responses in normal stromal fibroblast^[20], at least partly through Notch-dependent mechanisms^[21]. In another study, CAFs expressed vascular endothelial growth factor in presence of hypoxia inducible factor 1 α /G-protein estrogen receptor (HIF-1 α /GPER) signaling, suggesting a role for these cells in hypoxia-dependent tumor angiogenesis^[22]. Under the same conditions, CAFs were shown to express Notch molecules^[23], which promotes cancer cell survival, proliferation^[24,25], as well as angiogenesis^[26]. In addition, Luga *et al.*^[27] showed that CAFs release exosomes, which stimulate invasiveness and malignant cell metastasis *via* a Wnt11-dependent mechanism. On the same hand, CAFs induced phenotypical changes in adipocytes resulting in the generation of fibroblast-like cells [adipocyte-derived fibroblasts (ADF)], which in turn increased migratory abilities of metastatic cells by releasing high levels of collagen I and fibronectin^[28]. Notably, CAF-induced ADF phenotype generation was mediated by reactivation of the oncogenic Wnt/ β -catenin pathway in the latter cells in response to Wnt3a produced by the cancer cells, suggesting CAFs and ADFs as potential therapeutic targets in metastatic breast cancer. Furthermore, CAFs may promote breast cancer initiation and progression to metastasis *via* tumor- $\alpha\beta 1$ integrin signaling^[29] and fibroblast growth factor signaling^[30], as well as malignancy orchestration and tumor stroma reprogramming through activation of heat shock factor 1^[31], a transcriptional regulator.

Interestingly, Capparelli *et al.*^[32,33] have hypothesized that senescent fibroblasts may promote tumor growth through an autophagy-dependent mechanism termed as “autophagy-senescence transition”. In order to test such hypothesis, these authors introduced autophagy genes such as *bnip3*, *ctsb* or *ATG16L1* in immortalized human fibroblasts that resulted in the induction of a constitutive autophagic phenotype (characterized by mitophagy, aerobic glycolysis, L-lactate and ketone body production) with senescence features associated with increased β -galactosidase activity, increased level of cyclin depen-

dent kinase inhibitor (CDKI) p21, and cellular hypertrophy. Interestingly, “autophagic-senescent” fibroblasts were able to induce tumor growth and metastasis independently of angiogenesis, with stronger effects (up to 11-fold) in autophagic fibroblasts producing large amounts of ketone bodies. These observations were confirmed *in vivo*, as the lysosomal enzyme and biomarker of senescence, β -galactosidase, was also found in human breast cancer stroma. A recent *in vivo* study revealed the ability of CAF autophagy and senescence to promote tumor growth and metastasis increasing the rate of glycolysis and enhancing the generation of mitochondrial fuels including bodies^[33] in a compartment-specific fashion, thus supporting the role of CAFs to metabolically regulate tumorigenesis. In this study, the injection of the antidiabetic molecule along with peroxisome proliferator-activated receptor gamma (PPAR γ), known to stimulate glycolysis and pro-autophagy, into stromal cells enhanced the growth of co-injected breast cancer cells by 60%, whereas PPAR γ injection in cancer cells reduced the growth of breast cancer cells by 40%^[34].

Tumor-associated macrophages

TAMs infiltration into neoplastic tissues is an important negative prognostic factor^[35,36], and a hallmark of triple negative breast cancer^[37], a chemoresistant subtype of breast cancer^[38,39]. Overall, emerging evidence suggests that TAMs are major player in anticancer drug resistance in breast cancer. For instance, Yamashina *et al.*^[40] recently reported that cancer stem-like cells originating from chemoresistant tumor promote macrophage colony-stimulating factor production *via* an interferon regulatory factor 5-dependent mechanism, and transform recruited CD14(+) monocytes in tumorigenic M2-macrophages (immunoregulatory), probably through CXCR3 downregulation^[41]. Interestingly, the differentiation inducer dimethyl sulfoxide exerted antitumor effects in a mouse breast cancer model (4T1) possibly by inducing M1-phenotype in TAMs^[42].

Furthermore, TAMs may promote carcinogenesis and metastasis *via* Wnt signaling, which mediates the angiogenic switch and metastatic processes in breast cancer^[43,44]. Notably, TAMs release high levels of the Wnt family ligand Wnt7b^[45], and cancer stem-like cells may trigger the metastatic effect of TAMs through enhancement of the β -catenin pathway *via* vitamin D receptor suppression by tumor necrosis factor alpha^[46]. In addition, *in vivo* and *in vitro* studies supported a pivotal role for Wnt 5a signaling in TAMs-induced metastasis^[47,48], and a strong correlation was found between Wnt5a expression in malignant cells and the number of CD163(+) M2-macrophages^[49]. In a relatively recent study investigating the potential of the phosphodiesterase type 5 inhibitor (vasodilator) drug dipyridamole in xenograft mice, anticancer effects were mediated at least partly by decreasing β -catenin cytosolic levels^[50]. Altogether, these findings implicated TAMs as a key links between chemoresistance and tumorigenic activities of cancer stem-like cells, and thus, positioning TAMs as potential therapeutic targets

for breast cancer. Figure 1 shows the main signaling pathways currently in use for targeted breast cancer therapy, as well as some possible new targets.

NOTCH SIGNALING

Notch family of molecules

The Notch family of membrane bound receptors and ligands regulate several cell processes including cell invasion, survival and apoptosis, *via* the Notch signaling pathway. The pathway comprises four receptors (Notch1 through Notch4) and five Notch ligands (Delta-like 1, 3, and 4, and Jagged1 and 2). Notch ligands include an extracellular domain containing multiple epidermal growth factor (EGF)-like repeats and an extracellular DSL where ligand binding occurs, and an intracellular domain with a PDZ-binding motif at C-terminal domain^[51,52]. Notch receptors are also made of an extracellular and an intracellular domain covalently linked. Notch receptor extracellular domain also contains EGF-like repeats (26-29 depending on the Notch receptor), whereas Notch intracellular domain (NICD) presents with LIN12/Notch-related repeats preventing ligand-independent signaling, cysteine residues, and a C-terminal transactivation domain containing a PEST sequence with proteolytic activity.

Notch ligands are expressed on the plasma membrane of one cell and interact with Notch receptors on the plasma membrane of a neighboring cell, initiating the cleavage of the receptor by proteases [ADAM (a disintegrin and metalloprotease) and γ -secretase] that culminates in the release of the NICD^[53]. Released NICD translocate to the nucleus and forms a transcriptional activator complex with C-promoter binding factor 1/Suppressor of Hairless and Lag-1 (CSL) transcription factor. Together with cofactors like mastermind-like protein, NICD-CSL complex induces the transcription of cell fate key target genes such as *vegfr3* and, *notch1* that regulate angiogenesis and apoptosis, *p21* that regulates the cell cycle, as well as transcription factor genes such as the basic helix-loop-helix and hairy/enhancer of split/-related (*hes* and *hey*)^[54,55] (Figure 1).

Notch signaling as a therapeutic target

As already mentioned (section 2), Notch signaling is used by CAFs to promote cancer cell survival and proliferation. Early reports revealed that upregulation of Notch signaling suffices to transform normal breast epithelial cells in malignant cells *in vitro*, and that high levels of NICD are present in breast primary tumors^[56-59]. Notch carcinogenic effects are mediated *via* the silencing proapoptotic signaling pathways and growth-inhibitory molecules like TGF- β ^[58]. Notch-induced TGF- β silencing also promotes bone metastasis^[60,61]. In addition, Notch signaling, which is required for physiological angiogenesis, may also be a key player in neoangiogenesis^[62]. A Notch 3 addition of the lymphovascular embolus was reported in a xenograft model of inflammatory breast carcinoma, a subtype of breast cancer whose hallmark is

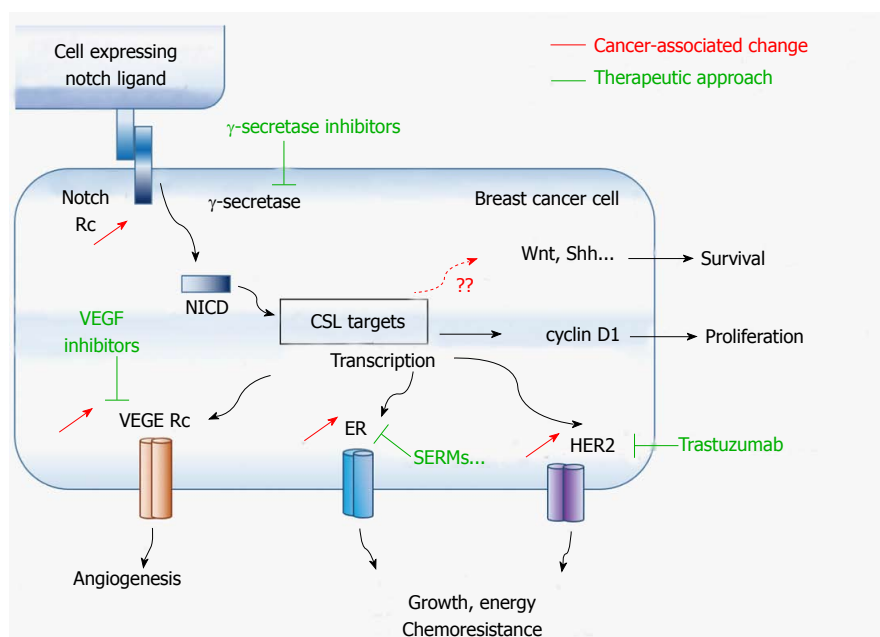


Figure 1 Notch signaling in breast cancer. In Notch-driven breast cancers, tumor cells and neighboring cells express Notch ligand and receptors. In presence of ADAM/TACE and γ -secretase enzymatic complex, Notch ligand-receptor interactions result in the release of Notch intracellular domain (NICD), which translocate to cell nucleus and activate CSL transcription factor. Target genes include signaling molecules involve in cancer cell survival, proliferation, angiogenesis, growth, energy metabolism, and chemoresistance. Inhibitors of many of these signaling molecules have been developed and are in use in various cancers, including g-secretase inhibitors, vascular endothelial growth factor inhibitors, estrogen signaling inhibitors, and HER2 inhibitors. ER: Estrogen receptor; HER2: Epidermal growth factor receptor 2; ADAM/TACE: A disintegrin and metalloprotease/tumor necrosis factor- α converting enzyme; CSL: CBF1/Suppressor of Hairless/LAG-1.

lymphovascular invasion^[63].

In vitro studies in estrogen receptor (ER)-negative breast cancer cells (MDA-MB-231) performed by Lee *et al.*^[64] revealed that Notch signaling up-regulates the transcription of the apoptosis inhibitor survivin. In another study, these authors showed that Notch-1-survivin functional gene signature is common in basal breast cancer^[65]. In addition, crosstalk between Notch and signaling pathways involved in cell growth were reported as well, including the estrogen receptor^[66], human epidermal growth factor receptor 2 (HER2)^[67], and the metabolic signaling pathways phosphatidylinositol 3-kinase (PI3K)/ protein kinase B (Akt)/mammalian target of rapamycin (mTOR)^[68,69] and MAP kinase/ERK^[70,71]. Interestingly combined targeting of Notch and EGFR signaling suppressed chemoresistance in a basal-like breast cancer *in vivo* model^[72], suggesting that co-targeting of Notch and associated pathways may represent a new avenue for overcoming chemoresistance (Figure 1).

Tumor initiating cells of tumors overexpressing HER2/neu also express high levels of Notch molecules, whose signaling is known to enhance HER2 expression^[73]. Chemoresistance to HER2+ breast cancers to trastuzumab, a monoclonal antibody against HER2, is associated with the overexpression of Notch-1 and its ligand Jagged-1^[74,75]. Similarly, cancer stem-like cells also achieve resistance against chemotherapy and radiotherapy *via* Notch signaling^[76], and targeting of this signaling pathway reduces the stem-like population^[77]. The γ -secretase inhibitor MRK-003 induced long-term recurrence-free survival in a transgenic mouse model of HER2+ breast cancer^[78]. Similarly, co-targeting of Notch and HER2

signaling pathways prevented breast tumor recurrence in orthotopic breast tumor xenograft using trastuzumab-resistant BT474 cells^[79].

Platelet-derived growth factor-D, another marker of breast cancer poor prognosis, may increase breast tumor aggressiveness by activating Notch and NF- κ B signaling pathways^[80]. Furthermore, Notch-1 and Notch-4, established bio-markers of the chemoresistant breast cancer subtype^[81], were reported as novel transcriptional targets in triple negative breast cancer^[82,83]. Jagged1/Notch4 signaling was shown to induce epithelial-to-mesenchymal transition^[84]. Notch signaling was also reported as a mechanism of resistance to PI3K inhibitors^[85] and hormone therapy^[86].

Clinical evaluation of Notch signaling targeting

Notch signaling inhibitors have a promising clinical efficacy as they abrogate HER2-Notch axis of chemoresistance. Notch silencing by γ -secretase inhibitors (GSIs) inhibited the proliferation of breast cancer cells partly by causing cell cycle arrest and apoptosis^[76], and by sensitizing chemoresistant breast cancer cells to the BH3 mimetic ABT-737^[87]. Notably, GSIs induce toxicity to breast cancer both *in vitro* and *in vivo* models, however mechanisms of such cytotoxicity are complex and may involve proteasome inhibition and downregulation of Bax and Bcl-2^[88,89].

Following encouraging pre-clinical studies^[83,90,91], the oral gamma secretase inhibitor R04929097 recently entered phase- I trial in patients with advanced solid tumors. Early reports of combination therapies with the kinase inhibitor temsirolimus^[92], the antimetabolites of the

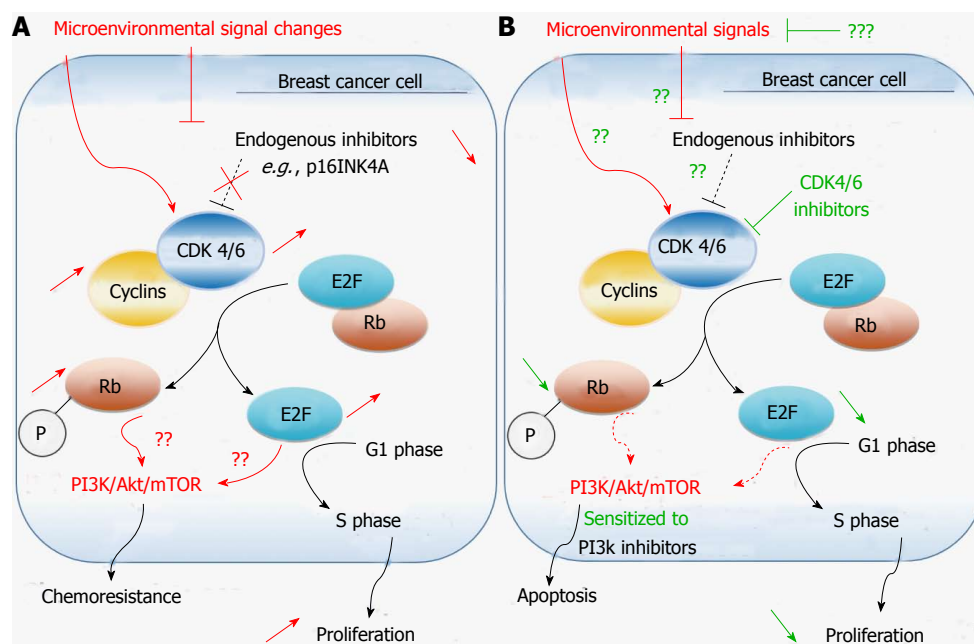


Figure 2 CDK4/6 signaling in breast cancer. A: Cyclin dependent kinases (CDK) 4/6 signaling is overexpressed in breast cancer. Such overexpression, which results from the silencing of CDK endogenous inhibitors, participate directly to cancer cell proliferation by triggering G1-S transition, and indirectly to chemoresistance via a PI3K/Akt/mTOR-dependent mechanism; B: CDK4/6 inhibitors sensitize chemoresistant cells to PI3K inhibitors and various other anticancer agents. PI3K: Phosphatidylinositol 3-kinase.

pyrimidine analog family gemcitabine (PHL-078/CTEP 8575)^[93] or cediranib (PJC-004/NCI 8503) revealed that the combinations were safe and promising in breast, tracheal, and pancreas cancer patients. However, anemia, diarrhea, fatigue, hypertension, neutropenia, and nausea were observed, among other side effects. GSI reported side effects seem to be mediated primarily through proteasome inhibition^[88,94]. Thus, CSL inhibition, which was reported to mediate a more effective inhibition of Notch-dependent carcinogenic processes than GSIs^[95], may represent a less toxic approach for Notch signaling targeting.

Another GSI, PF-03084014, also presented promising results in breast xenograft models^[96], with gastrointestinal toxicity easily abrogated by glucocorticoids^[97]. Other promising pre-clinical observations included a synergistic effect with the antimitotic drug docetaxel in breast cancer^[98], colorectal cancer^[99], and metastatic pancreatic cancer^[100] models. Antiangiogenic effects were also reported in combinations with the tyrosine kinase inhibitor sunitinib in solid tumors^[101], whereas in chronic lymphocytic leukemia cells combinations with the nucleoside metabolic inhibitor fludarabine inhibited angiogenesis as well as migration and invasion of Notch 1-mutated cancer cells^[102,103]. PF-03084014 therefore appears as an appealing GSI for both solid and blood cancers and may be a good targeted-therapy drug in breast cancer.

CDK

CDKs, cyclins and CDKI

Cyclins, CDK inhibitors (CDKIs, *e.g.*, p16INK4, p15INK4B, p18INK4C, p21WAF1/CIP1^[104,105]) and CDKs are the three key classes of regulatory molecules that deter-

mine cell cycle progression through the G0-G1-S-G2 and M phases^[106,107]. Numerous CDKs are found in eukaryotic cells, of which some are pivotal cell cycle regulators, such as CDK1/2/4/6 (Figure 2). CDKs (catalytic subunits, heterodimeric serine/threonine kinase class) associate with cyclins (regulatory subunits) to form an active catalytic complex favoring G1/S cell-cycle progression in mitosis. For instance, CDK1/A2 or CDK1/B1 complexes trigger mitosis in mammalian cells by phosphorylating downstream cell cycle regulatory proteins^[108]. Other CDKs are involved in the regulation of cellular transcription, such as CDK7-11^[107,109]. A recent proteomic analysis of the CDK family in human cells has identified a CDK5 complex as a key regulator of non-neural cell growth and migration factor^[110].

CDK involvement in breast cancer

Early and emerging evidence suggests that cyclin D1 promotes breast tumorigenesis^[111,112]. CDK1 activity was recently reported as a powerful predictor of taxane chemosensitivity, indicating a role for CDK1 in breast tumorigenesis^[111]. Notably, taxanes are the drug class most used for breast cancer pre-operative chemotherapy; they induce apoptosis in malignant cells by stopping their replication^[113,114]. Moreover, studies investigating genes that are synthetically lethal in Myc-dependent cancer identified numerous CDKs as Myc synthetic-lethal genes^[115-117]. Interestingly, in one of such studies CDK1, but not CDK2 or CDK4/6 was selectively lethal to Myc-dependent breast cancer cells^[117]. This observation indicates that targeting CDK1 may induce apoptosis in Myc-dependent cancers, where Myc drives cancer cell growth and cycle progression^[118]. Increases in activities and levels of other CDK complexes were also reported in breast cancer

primary tumors and experimental models, including CDK4/6 and cyclin E/CDK2 complexes^[119-121]. The occurrence of cyclin E/CDK2 proteolytic cleavage products associates with poor clinical outcome in breast cancer patients and increases tumorigenicity in experimental models at least partly by promoting stem-like properties of tumor cells^[120]. Transcriptional regulator CDK8 targeting was also recently reported to inhibit both the proliferation and the migration of breast cancer cells^[122]. In addition, *BRC42* gene, whose aberrant activating mutations associate with familial breast cancer^[123,124], was reported to induce genomic stability in malignant cells through a CDK-dependent mechanism^[125].

A link between the cell cycle and steroid hormone metabolism involving CDK4/6 was recently uncovered in breast cancer primary tumor cells^[126]. In this study, malignant cells appeared to control the activity of steroid metabolic enzymes, *i.e.*, the expression of steroid hormone receptors (including ER), by alteration of CDK4/6-levels (overexpression of CDK4 and decrease of its homolog CDK6). Such mechanism may play a pivotal role in the carcinogenesis and chemoresistance of steroid hormone-dependent cancers. In another recent study the newly synthesized compound KU004 that had a potent anticancer effect by targeting HER2 induced a decrease in CDK4 expression^[127]. On the same hand, CDK 4/6 inhibitors sensitized *PIK3CA* mutant breast cancer to PI3K inhibitors in a xenograft study^[128] (Figure 2), further suggesting a role for CDK4/6 imbalance in breast tumorigenesis.

CDK inhibitors

CDK4/6 inhibitors are more efficient and less toxic anti-neoplastic agents than molecules targeting other CDKs^[129]. The selective cyclin D kinase 4/6 inhibitor palbociclib (PD-0332991) is currently entering phase III trial for ER+ breast cancer patients, following encouraging results in progression free survival in phase II trials^[130]. Using the bioluminescence imaging technology, an early study in xenograft models displaying metastatic progression revealed powerful antimetastatic effects, comparable to avastin, and docetaxel effects^[131]. In addition, palbociclib, preferentially inhibited the proliferation of luminal ER+ breast cancer cell lines *in vitro*^[132], suppressed malignant cell proliferation in approximately 85% of cases irrespective of ER+/- or HER2+/- statuses^[133]. Furthermore, palbociclib induced growth arrest in hormone-resistant MCF-7 breast cancer cells by a mechanism consistent with cellular senescence^[134]. This observation is not surprising considering the functional link between tumor microenvironment carcinogenic activity, ageing, and autophagy discussed above (section 2.1), and indicate that the drug may also affect metabolic processes in CAFs and stem-like tumor cells^[133,134].

Chemoresistance to CDK4/6 inhibitors has been reported^[133,135]. Analyses of primary tumor cells of cases resistant to CDK4/6 inhibitors showed that these cells lack the tumor suppressor retinoblastoma protein (RB)^[133],

which is necessary for CDK4/6 control of the cell cycle restriction point^[135]. Interestingly, RB-deficient chemoresistant breast cancers, such as RB-deficient triple negative breast cancers, are more sensitive to the metabolic inhibitor of the folate analog family methotrexate and to the anthracycline topoisomerase inhibitor doxorubicin compared to RB+ cell lines^[136], indicating that combination therapy may improve CDK4/6 inhibitor response in resistant cases. However, a report by Roberts and colleagues cautioned against the use of these agents in combination with DNA-damaging drugs (*e.g.*, doxorubicin, carboplatin), considering the potential genotoxic side effects^[129]. The dangers that may result from such combination also emerged in other pre-clinical studies^[137,138].

The CDKI dinaciclib (MK-7965), which selectively binds to the ATP site of CDKs and acts as a protein-protein inhibitor of bromodomains^[139,140], also displayed encouraging anticancer properties in pre-clinical studies in human cancer models^[141,142]. The drug recently entered phase III in leukemia^[139] and phase II trial in solid cancers. The drug is well tolerated in monotherapy, but revealed an antitumor activity whose efficacy was not superior to the nucleoside metabolic inhibitor capecitabine in a phase II trial in advanced breast cancer patients^[143]. Comparable observations were reported in non-small cell lung cancer where the drug was compared with the protein kinase inhibitor erlotinib^[144]. Similar combination therapy studies in progress for breast cancer^[143,144] may provide alternative strategies for breast cancer therapy.

OTHER EMERGING THERAPEUTIC TARGETS

Wnt signaling

A number of reports have suggested that Wnt signaling pathway, which is normally involved in embryonic induction and cell fate^[145,146], is aberrantly activated in blood cancers^[147-149] and solid cancers, such as head and neck, lung, gastrointestinal, and breast cancer^[27,150-155]. Wnt5a and Wnt11 are major players in macrophage-induced malignant invasion in metastatic breast cancer^[27,151], and several breast tumors constitutively release-inducible Wnt ligands^[156]. In addition, the naturally occurring pentacyclic triterpenoid ursolic acid, which is known to exert anti-tumor activity in various solid cancers including breast cancer, may act through inhibition of canonical (Wnt/ β -catenin) signaling^[150]. Similarly, the natural plant polyphenol rottlerin was reported to inhibit Wnt/ β -catenin signaling in cancer cells by promoting the degradation of Wnt co-receptor LRP6 (low density lipoprotein receptor-related protein 6)^[157]. Such inhibition resulted in cell death in various cancer cell lines, including MDA-MB-231 and T-47D breast cancer cells. Salinomycin, another novel LRP6 inhibitor, induced comparable effects in breast and prostate cancer cell lines, by inhibiting both Wnt/ β -catenin and PI3K/Akt/mTOR signaling^[158].

The development of specific Wnt inhibitors is in

progress. Recently, a specific inhibitor of Porcupine (PORCN, an O-acyltransferase required for the secretion of Wnt ligands^[159]) termed as LGK974 was developed. LGK974 displayed potent anticancer properties in *in vitro* and *in vivo* models of breast cancer and pancreatic adenocarcinoma mediated by reduction of the transcriptional expression of Wnt target genes^[147,160]. However, another recent report revealed that Wnt signaling molecules are differentially expressed in breast cancer clinical subtypes and in cancer stem-like cells, indicating that the development of more specific Wnt-targeted therapies in breast cancer may be necessary^[161]. Wnt signaling was also reported a major role in malignant cell acquired resistance to classical chemotherapy, including resistance to tamoxifen^[162], and in chemoresistant cells from triple negative breast cancer patients^[163], suggesting the potential of Wnt inhibitor combination therapies.

Shh signaling

Early studies have suggested that Sonic Hedgehog (Shh) overexpression, mediated by both NF- κ B up-regulation and *shh* promoter hypomethylation in breast cancer^[164], is a critical event in the development of various solid cancers^[165-167]. For instance, Shh signaling was reported to promote the survival of cancer epithelial cells, but not their normal counterparts^[168]. Targeting of Shh transcription activator Gli1 enhanced apoptosis and attenuated migration in inflammatory breast cancer cells^[169]. In addition, Shh non-classical activation was reported as a multidrug resistance enhancer, including resistance to Smo inhibitors^[170], suggesting that targeting these pathways specifically may abrogate the associated chemoresistance.

Smo inhibitor anticancer drug cyclopamine, which inhibits Shh signaling by antagonizing its downstream target Smo, is metabolically stable and is currently investigated for the treatment of various cancers^[171-173]. The chemotherapy drug paclitaxel used in combination with cyclopamine was shown to antagonize chemoresistant breast cancer cells both *in vivo* and *in vitro*^[174], suggesting Shh signaling as a candidate for targeted therapy in chemoresistant cancer cells. Similarly, cyclopamine also sensitized chemoresistant tumor cells to taxane drugs in ovarian cancer^[175], another hormone-related cancer. Not surprisingly, Shh targeting was reported as a therapeutic option in endocrine-resistant breast cancer due to its ability to sensitize PI3K/AKT signaling-induced tamoxifen chemoresistant malignant cells^[176].

Notably, ER- α physiologically regulates non-canonical Shh signaling in the mammary gland, and is essential for mammary gland morphogenesis at puberty^[177,178]. However, Gli1 expression also enhances migration and invasion of malignant cells in ER α -negative and triple negative breast cancers, where it represents a predictor of poor prognosis^[179]. These observations indicate that Shh signaling involvement in breast cancer cells is complex and therefore targeting Shh in chemoresistant cancer therapy can also compromise its normal physiological function.

FUTURE DIRECTIONS:

PERSONALIZED-BASED THERAPY AND EPIGENETIC TARGETS

Personalized-based therapy

The major challenges in breast cancer treatment include resistance to chemotherapy, hormone therapy and even targeted therapy (Table 1), which underline the need for developing novel targeted therapies. Although the main molecular events driving cancer involve the activation of proto-oncogenes or the inactivation of tumor suppressors, deregulation of various signaling intermediates and metabolic factors have been well documented^[72,77,82,83,149,161]. The events triggering cancer development affect proto-oncogenes such as Notch, Wnt, and Shh, which are the developmental genes driving embryonic induction and organogenesis during fetal life. These genes, whose expression is normally transcriptionally reduced or silenced in most adult tissues (except stem-like cells) by regulator molecules, are aberrantly overexpressed in cancer cells, conferring them stem-like properties^[72,77,82,83,149,161].

Concomitantly, neoplastic tissue growth is fuelled by the upregulation and overexpression of receptors such as HER2, ER and, IGF-1R^[70,71,180], the upregulation and/or activation of signaling molecules associated with cell proliferation^[111,112], cell migration^[181,182], oxidative stress, hypoxia and neoangiogenesis^[22,26], all which are characteristic of tumor microenvironment. Thus, the complete characterization of all these tumor promoting events will pave the way for the development more efficient and less toxic anticancer drugs. Computational causal network models aimed at improving the current understanding of signaling molecule interactions in breast cancer, which will allow the determination of specific subsets of patients susceptible to a given therapeutic approach, are currently in development^[156,183]. Although the complexity of such networks makes this effort challenging, nonetheless, the development of such tool would allow implementation of a highly efficient personalized-based therapy in breast cancer.

Epigenetic changes drive tumorigenesis

Epigenetics describes heritable alterations in gene expression patterns that do not alter the primary DNA sequence, but play critical roles in normal differentiation and development. Epigenetic alterations include modifications such as DNA methylation, histone modifications and nucleosome remodeling. The plasticity and reversibility of epigenetic events enable a better control of the dynamism of cellular processes. However, deregulation of the normal epigenetic patterns can lead to aberrant expression of cell growth regulatory genes that can culminate in cancer. Epigenetic factors affect gene expression both pre- and post-transcriptionally and probably account for the high inter-individual variability in clinical course and treatment outcome of both blood and solid cancers^[184,185]. There is ample evidence linking

Table 1 Current therapeutics for breast cancer

Drug	Trade name	Class	Anticancer mechanism
Classical chemotherapy			
Methotrexate	Abitrexate®, Mexate®, Folex®	Antimetabolites, folate analogs	Folate receptor competitive antagonist ^[218]
5-fluorouracil	Adrucil®, Efudex®, Fluoroplex®, prodrug capecitabine/Xeloda®	Antimetabolite, pyrimidine analogs	Inhibition of the phosphatase and tensin homolog thymidylate synthase ^[219]
Gemcitabine hydrochloride	Gemzar®	Anthracycline	Deoxyribonuclease inhibitor ^[220]
Doxorubicin hydrochloride	Adriamycin®		
Epirubicin hydrochloride	Ellence®		
Pamidronate disodium	Aredia®		
Cyclophosphamide	Clafen®, Cytoxan®, Neosar®	Nitrogen-containing bisphosphonate	Inhibition of farnesyl pyrophosphate synthase activity ^[221]
		Nitrogen mustard alkylating agent	Inhibition of DNA replication by interacting with the alkyl group of DNA guanine base ^[222]
Paclitaxel	Abiraxane® Taxol®	Taxanes	Microtubule Inhibitors ^[223,224]
Docetaxel	Docetad®, Taxotere®		
Ixabepilone	Ixemptra®	Epothilone B analog	
Targeted therapy			
Everolimus	Afinitor®	mTOR inhibitor	Silencing of PI3K/ Akt/mTOR signaling ^[225]
Trastuzumab	Herceptin®	HER2 inhibitor	Anti-HER2 monoclonal antibodies ^[226,227]
Pertuzumab	Perjeta®	Antibody-drug conjugate Dual tyrosine kinase inhibitor	HER2 inhibitor and cytotoxic agent ^[228]
Ado-Trastuzumab Emtansine	Kadcyla®		
Lapatinib ditosylate	Tykerb®		
Hormone therapy			
Toremifene	Fareston®	Selective ER modulator ER antagonists	Silence ER signaling ^[230,231]
Fulvestrant	Faslodex®		
Tamoxifen citrate	Nolvadex®	Aromatase inhibitors	Inhibit estrogen synthesis ^[232-234]
Anastrozole	Arimidex®		
Exemestane	Aromasin®		
Letrozole	Femara®	GnRH agonist Progesterone derivative	Progestational and antigonadotropic effects ^[235]
Goserelin acetate	Zoladex®		
Megestrol acetate	Megace®		

PI3K: Phosphatidylinositol 3-kinase; Akt: Protein kinase B; HER: Epidermal growth factor receptor 2.

the etiology of breast to abnormal genetic and epigenetic events^[180,186,187]. Cancer-specific DNA methylation changes and well as dysregulation of histone modification have been characterized as contributors to breast cancer development. Progress in our understanding of epigenetics mechanisms in breast cancer have led to the identification of novel therapeutic targets. Recent therapeutic strategies involving the use of epigenetic agents alone or in combination with chemotherapy and/or endocrine therapy are showing promising results in breast cancer patients including chemoresistant cases^[186,188].

The technological breakthrough of “omics era” has allowed the development of high-throughput sequencing technology allowing both global and comprehensive investigations of the interactome, the epigenome, and the transcriptome (*i.e.*, active signaling pathways, cascades of pre- and post-translational changes affecting specific genes, and changes in gene expression)^[189-191] at individual level. Epigenetic alterations in cancer constitute appealing therapeutic targets due to their pivotal roles in disease initiation, progression, and chemoresistance, and to their reversibility. For instance, chemoresistance to the ER antagonist fulvestrant is mediated by epigenetic modulation (more specifically hSWI/SNF-mediated chromatin remodeling) of GPER and CDK6 expression^[192], suggesting that adjuvant therapy targeting SWI/SNF activity may induce apoptosis in resistant cancer cells. SWI/SNF

tumor-dependency has also been reported in other solid cancers and in leukemias^[193,194].

Epigenetic targets in breast cancer: histone deacetylation and DNA hypermethylation

Studies have shown that the transcriptional expression of various signaling molecules associated with breast cancer and other cancers may result from selective epigenetic silencing of regulator genes mediated by histone deacetylation and gene promoter (DNA) hypermethylation^[195-197], among other potential epigenetic mechanisms^[186,198]. For instance, the reduction in ER expression observed in various chemoresistant breast tumors may be mediated by epigenetic silencing (*e.g.*, *erbB1* silencing)^[199]; and some histone deacetylases (HDACs) such as HDAC3/8 were reported to play pivotal regulatory roles in the proliferation of normal and MDA-MB-231 cells^[200].

Data from numerous pre-clinical *in vivo* and *in vitro* studies support the potential of DNA methylation status targeting in breast cancer. Both the HDAC inhibitor (HDACI) trichostatin A and the DNA methyltransferase (DNMT) inhibitor (DNMTI) deoxycytidine (5-aza-2'-deoxycytidine) induced apoptosis in various breast cancer cell lines^[201-205]. The HDACI Romidepsin (FK-288) eliminated both primary and metastatic tumors in combination with Paclitaxel in the Mary-X pre-clinical model of inflammatory breast cancer^[206]. The green tea-derived

anticancer molecule epigallocatechin-3-gallate suppressed invasiveness in MDA-MB-231 and MCF-7 breast cancer cells by silencing matrix metalloproteinase 2 (MMP2) and MMP-9 and inducing TIMP-3 through increased activities of the enhancer of zeste homolog 2 and HDACs^[207]. Suberoylanilide hydroxamic acid, another naturally occurring HDACI, restored radiosensitivity and suppressed breast cancer lung metastasis *in vitro* and *in vivo*^[208].

The HDACI Vorinostat sensitized mesenchymal-like triple-negative breast cancer cell lines to hormone therapy by reactivating ER α ^[209] and PI3K/Akt/mTOR signaling sensitivity^[210], corroborating the role of epigenetic alterations in chemoresistance development in breast tumors. Furthermore, the HDACI abexinostat induced cancer-like stem cells differentiation in 16 breast cancer cell lines^[211]. Because of these interesting observations, the HDACIs belinostat, panobinostat, and vorinostat, previously used only in blood cancers, have entered phase I and II clinical trials in solid tumors, such as lung, prostate, gastrointestinal, ovarian and breast cancer, where they are showing encouraging results (for review see^[212]). Various DNMTI are also showing encouraging responses in metastatic and chemoresistant breast cancers in monotherapy and in combination therapies in phase I and II trials^[213-217].

CONCLUSION

Targeted therapies are associated with reduced adverse effects and better outcome. Tumor microenvironment cells such as cancer-associated fibroblasts and tumor-associated macrophages undergo aberrant genetic and epigenetic changes that trigger the overexpression of signaling molecules promoting neoplasia and neoplastic tissue survival. Many therapeutic targets have emerged. They include Notch, CDKs, mTOR, Wnt, and Shh, whose inhibitors are showing promising results in ongoing clinical trials, both in monotherapy and in combination therapy. Similarly, epigenetic drugs are also showing encouraging results in breast cancer, particularly in advanced and chemoresistant cases. New technological advances will enable the identification of precise alterations affecting the interactome, transcriptome, and the epigenome, leading to the design of more specific tailored therapies. Such therapeutic approach may also be beneficial in the treatment of chemoresistant breast cancers.

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Antimicrobial resistance in *Acinetobacter baumannii*: From bench to bedside

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Abstract

Acinetobacter baumannii (*A. baumannii*) is undoubtedly one of the most successful pathogens in the modern healthcare system. With invasive procedures, antibiotic use and immunocompromised hosts increasing in recent years, *A. baumannii* has become endemic in hospitals due to its versatile genetic machinery, which allows it to quickly evolve resistance factors, and to its remarkable ability to tolerate harsh environments. Infections and outbreaks caused by multidrug-resistant *A. baumannii* (MDRAB) are prevalent and have been reported worldwide over the past twenty or more years. To address this problem effectively, knowledge of species identification, typing methods, clinical manifestations, risk factors, and virulence factors is essential. The global epidemiology of MDRAB is monitored by persistent surveillance programs. Because few effective antibiotics are available, clinicians often face serious challenges when treating patients with MDRAB. Therefore, a deep understanding of the resistance mechanisms used by MDRAB can shed light on two possible strategies to combat the dissemination of antimicrobial resistance: stringent infection control and

antibiotic treatments, of which colistin-based combination therapy is the mainstream strategy. However, due to the current unsatisfying therapeutic outcomes, there is a great need to develop and evaluate the efficacy of new antibiotics and to understand the role of other potential alternatives, such as antimicrobial peptides, in the treatment of MDRAB infections.

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Key words: *Acinetobacter baumannii*; Antibiotic resistance; Epidemiology; Genomics; Infection control

Core tip: With the current rapid increase in the numbers of studies on *Acinetobacter baumannii* (*A. baumannii*), the complexity of the entire picture regarding how this superbug copes with its environment and influences human beings is gradually being understood. By conducting a thorough review of this topic, this paper aims to present the relevant literature regarding the antimicrobial resistance of *A. baumannii* and the currently available treatment options for *A. baumannii* infections to highlight possible future research directions.

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INTRODUCTION

Species identification and current taxonomy

Acinetobacter spp. are glucose-non-fermentative, non-motile, non-fastidious, catalase-positive, oxidase-negative, aerobic Gram-negative coccobacilli^[1]. Since 1986, the taxonomy of the genus *Acinetobacter* has been modified several times. Currently, the original single species clas-

sification of *Acinetobacter calcoaceticus* (*A. calcoaceticus*) has been abandoned, and at least 34 genomic species can be distinguished within the genus *Acinetobacter*, 23 of which have been assigned species names^[2]. The challenge in the taxonomy of *Acinetobacter* is due to the clusters of closely related species that are difficult to distinguish using phenotypic traits and chemotaxonomic methods. The *A. calcoaceticus*-*Acinetobacter baumannii* (*A. baumannii*) complex, comprising *A. calcoaceticus*, *A. baumannii*, and the genomic species 3 and 13TU, is the most well-known example^[3]. Because the antibiotic susceptibilities and clinical relevance of the different genomic species are significantly different^[4-7], genomic methods for *Acinetobacter* species identification are necessary. A number of genomic fingerprinting methods have been proposed, including pulsed-field gel electrophoresis (PFGE); ribotyping; polymerase chain reaction (PCR)-based fingerprinting techniques, such as random amplified polymorphic DNA analysis; repetitive extragenic palindromic sequence-based PCR (rep-PCR); amplified ribosomal DNA restriction analysis; RNA spacer fingerprinting; and amplified fragment length polymorphism analysis^[8]. In addition, new methods, such as 16S-23S ribosomal intergenic spacer, 16S rRNA gene, *rpoB* gene and *gyrB* gene sequence analyses, have been developed for *Acinetobacter* species identification^[9,10].

Common typing methods in outbreak investigations

Of all of the *Acinetobacter* species, *A. baumannii* is the most important member associated with infections in clinical practice and causes most of the reported outbreaks. In addition to chart review and statistical epidemiology, some DNA fingerprinting methods are valuable in outbreak investigations and strain discrimination. Rep-PCR, PFGE, and multilocus sequence typing (MLST) have all been used in previous studies. Rep-PCR has been proven to be a useful and expedient method for the epidemiological characterization of *A. baumannii* nosocomial outbreaks^[11]. Rep-PCR has also been used as a tool for determining species lineages of *A. baumannii* in a hospital^[12] and for differentiating pan-European, multi-resistant *A. baumannii* clone III from clones I and II^[13]. Despite the inter-laboratory variability of rep-PCR, this method has the advantage of being faster to perform than PFGE and MLST. The intra-laboratory clustering of *A. baumannii* has been shown to be well conserved^[14] and to correlate well with PFGE^[15] or MLST results^[16], demonstrating the robustness of rep-PCR. We have found one rep-PCR major cluster (84%) of *A. baumannii* carrying a class I integron that spread among four regional hospitals in northern Taiwan^[17]. However, PFGE is still considered the gold standard for typing outbreak-related isolates of *A. baumannii*^[18-21], whereas MLST provides a high level of resolution and is an excellent tool for studying the population structure and long-term epidemiology of *A. baumannii*^[22]. Recently, the *A. baumannii* MLST database (<http://pubmlst.org/abaumannii/>) was developed for the BIGSdb genomics platform^[23] to assist the broader community in elucidating the structure and function of this

microorganism.

Clinical manifestations

A. baumannii, named after Paul Baumann, is ubiquitous in soil and water^[24]. Previously, *A. baumannii* was regarded as a low-virulence commensal bacterium. However, it has become a successful pathogen^[25] and has emerged as a major cause of healthcare-associated infections, most of which have occurred in critically ill patients in the intensive care unit (ICU) setting^[26]. In recent decades, infections caused by *A. baumannii* have also occurred outside the ICU or in trauma patients after natural disasters, and they have even affected patients with co-morbidities in the community^[27]. Reports of community-acquired *Acinetobacter* infections have increased over the past decade^[28]. Several different types of infections, including pneumonia, urinary tract infections, bacteremia, wound infections and even meningitis, are caused by this organism^[29]. These infections often occur in older patients, many of whom have chronic underlying diseases and have previously received antimicrobial treatment^[30,31]. The mortality of patients with *A. baumannii* infections in hospitals and in the ICU has ranged from 7.8% to 23% and from 10% to 43%, respectively^[32].

Risk factors

In recent years, many studies have reported the risk factors for acquiring *A. baumannii* infections and have particularly focused on those caused by multidrug-resistant strains. The acquisition of MDRA is related to multiple factors, including environmental contamination and contact with transiently colonized healthcare providers^[33]. The independent risk factors for the acquisition of imipenem-resistant *A. baumannii* (IRAB) include a hospital size of > 500 beds, previous antimicrobial treatment, a urinary catheter, surgery^[34], previous ICU stay, and prior exposure to imipenem or third-generation cephalosporins^[35]. The only significant independent risk factor for the appearance of imipenem-resistant multidrug-resistant *A. baumannii* (MDRA) in patients formerly infected with imipenem-susceptible MDRA is imipenem or meropenem exposure^[36]. For extensively drug-resistant *A. baumannii* (XDRA) infections, the prior use of imipenem, meropenem, piperacillin/tazobactam or fourth-generation cephalosporins and > 30 d of being bedridden have been found to be independent risk factors^[37]. A systematic review concluded that the acquisition and spread of *A. baumannii* appeared to be related to a large number of variables, the most important of which were deficiencies in the implementation of infection control guidelines and the use of broad-spectrum antibiotics^[38].

The risk factors that are associated with *A. baumannii* bacteremia are immunosuppression, unscheduled hospital admission, respiratory failure at ICU admission, previous antimicrobial therapy, previous sepsis in the ICU, and the invasive procedures index^[39]. Resistance to carbapenems, mechanical ventilation, and the presence of malignancy have also been found to be associated with high mortality rates in patients with *A. baumannii* bacteremia^[40].

Regarding ventilator-associated pneumonia caused by *A. baumannii*, the risk factors include neurosurgery, adult respiratory distress syndrome, head trauma, and large-volume pulmonary aspiration^[41]. Because various studies showed certain differences in the risk factors of acquiring drug-resistant *A. baumannii* bacteremia or pneumonia^[42-46], a separate investigation should be performed in each hospital setting to limit the spread of this pathogen^[38].

Virulence factors

Previously, *A. baumannii* was regarded as a low-grade pathogen; however, it contains virulence factors that enhance its bacterial toxicity and pathogenicity. A combined approach of genomic and phenotypic analyses led to the identification of several virulence factors, including extracellular components with hemolytic, phospholipase, protease and iron-chelating activities, biofilm formation, surface motility, and stress resistance^[47]. The biofilm formation of *A. baumannii* facilitates its attachment to abiotic and biotic surfaces^[48], including those of medical devices and host tissues. The initiation and maturation of biofilms are related to pilus assembly and the production of the biofilm-associated protein (Bap), which is regulated by the two-component system BfmRS^[49]. The Bap protein plays a role in adhesion to human epithelial cells^[50], and the inhibition of this protein can prevent *A. baumannii* infection^[51]. In fact, in a multicenter cohort study, all catheter-related urinary or blood stream infections due to *A. baumannii* were caused by biofilm-forming strains^[52]. A 2D proteomic analysis of pellicle-forming *A. baumannii* isolates showed that overexpression of CarO, which is an OprD-homolog, siderophore iron uptake, and pili systems are involved in the development of biofilms^[53].

Iron uptake systems are essential to the survival and pathogenicity of bacteria, especially in the low-iron environment of the human host. *A. baumannii* grown under iron-limited conditions undergo major transcriptional changes of not only many iron acquisition-related genes but also of genes involved in motility^[54]. *A. baumannii* is well-equipped with metal homeostatic systems that are required for the colonization of a diverse array of tissues^[55]. Genome investigations have revealed wide distributions of endogenous siderophores in clinical *A. baumannii* isolates, arguing for their role in pathogenic capabilities^[47]. The zinc acquisition system has also been found in *A. baumannii*, which is required for efficient zinc uptake *in vitro* and full pathogenesis *in vivo*^[56].

A. baumannii adheres to human bronchial epithelial cells *in vitro*, and its prevalent European clone II has a relatively high capacity for adhering to these cells^[57]. Additionally, the K1 capsular polysaccharide has been shown to prevent *A. baumannii* from being phagocytized by macrophages, to optimize its growth in human ascites fluid and serum, and to enhance its survival in a rat soft tissue infection model^[58]. Moreover, several proteins have been implicated as possible virulence factors in *A. baumannii*. Omp38 induces the apoptosis of host cells^[59], the absence of the RecA protein decreases survival in

response to both heat shock and desiccation^[60], and the inactivation of phospholipase D diminishes *A. baumannii* pathogenesis^[61]. Importantly, the outer membrane protein A of *A. baumannii* (AbOmpA) is the most abundant surface protein that has been associated with the apoptosis of epithelial cells through mitochondrial targeting^[62]. AbOmpA is also the major nonspecific channel in *A. baumannii* and appears to be essential for this organism's high levels of intrinsic resistance to a number of antibiotics^[63]. *A. baumannii* can rapidly develop resistance to polymyxin antibiotics through the loss of the lipid A component of lipopolysaccharide^[64], which subsequently alters the expression of critical transport and biosynthesis systems associated with modulating the composition and structure of the bacterial surface^[65].

GLOBAL EPIDEMIOLOGY

Two key factors contributing to the significant and ubiquitous dissemination of *A. baumannii* in hospitals are the extent of its antimicrobial resistance and its environmental resilience^[66]. The extent of antimicrobial resistance is more severe in *A. baumannii* isolates from patients in Asian and European ICUs than from patients in American ICUs^[27], and significant increases in antimicrobial resistance were noted worldwide from 2004 to 2009. The highest resistance rates in 2009 were for ceftriaxone (83.6%), piperacillin-tazobactam (82.0%), and ceftazidime (80.3%) in the Middle East. Increases in resistance were noted for all antimicrobials in isolates collected from the Asia-Pacific Rim, ranging from a 19.1% increase in ceftazidime resistance to a 38.9% increase in levofloxacin resistance. Resistance also increased significantly in Africa (including piperacillin-tazobactam, ceftriaxone, cefepime, amikacin, meropenem, and levofloxacin resistance) and Europe (including piperacillin-tazobactam, ceftriaxone, ceftazidime, levofloxacin, amikacin, minocycline, meropenem, and cefepime resistance)^[67].

The first MDRAB isolate resistant to almost all available antibiotics in Taiwan was discovered in 1998^[68]. Since then, many MDRAB outbreaks have been reported in Taiwan^[69-72]. A Taiwanese surveillance report of antimicrobial resistance in 2000 found that 73% of *A. baumannii* isolates collected from 21 medical centers and regional hospitals were ceftazidime-resistant^[73]. Another study conducted during the same year at five major teaching hospitals in Taiwan showed that as many as 22% of *A. baumannii* isolates were not susceptible to imipenem^[74]. In 2012, the Taiwan Surveillance of Antimicrobial Resistance program showed that the prevalence of the IRAB complex increased from 3.4% in 2002 to 58.7% in 2010 and that of the XDRAB complex increased from 1.3% in 2002 to 41.0% in 2010^[75]. In addition, the proportion of healthcare-associated infections caused by carbapenem-resistant *A. baumannii* (CRAB) significantly increased, compared to infections by all *A. baumannii*, from 14% in 2003 to 46% in 2008 in Taiwan^[76]. The local spread of MDRAB has been demonstrated in five proximal hospitals in northern Taiwan, with resistance determinants

distributed widely in clonal and non-clonal isolates^[77].

In addition to its prevalence in Taiwan, MDRAB is also prevalent in hospitals in many areas of the world, including Korea^[78], Belgium^[79], Italy^[80], Iraq^[81], Israel^[82], Greece^[83] and America^[84]. Furthermore, a one-year study demonstrated that three clones of MDRAB had spread in hospitals in Brazil^[85]. In a single institution in Queensland, Australia, sequence type 92 (ST92) was the dominant sequence type and was present for 9 years^[86]. Additionally, clonal dissemination among three hospitals located in two different cities has been documented in China, indicating the epidemic potential of MDRAB^[87]. Both inter-institutional and intra-institutional transmission of a strain of *A. baumannii* is possible^[15]. Several multidrug-resistant clones can coexist endemically in one hospital for several years^[31,88], and the same clones often spread on a small scale within a short period of time^[31] or can be detected during an outbreak by a close survey of epidemic sources^[88]. Furthermore, such outbreaks can occur across national boundaries. For example, Wybo *et al*^[89] reported a MDRAB nosocomial infection involving approximately 20 patients in a university hospital in Belgium that was the result of a transfer of two patients from Greece.

In addition to the increasing importance of MDRAB in nosocomial infections, the increasing reports of outbreaks caused by CRAB in recent years have become another frightening reality. The imipenem resistance rate of *A. baumannii* from a worldwide collection between 2005 and 2009 reached resistance rates of greater than 50%^[90]. In Brooklyn, New York, citywide surveillance revealed that about 2 of every 3 isolates were resistant to carbapenem antibiotics^[91]. One predominant strain type of CRAB has established predominance after being introduced in a university hospital in Chicago in 2005^[21]. In addition, molecular epidemiological investigations of sequential outbreaks of *A. baumannii* in an ICU showed the emergence of carbapenem resistance in Italy from 1999 to 2002^[19]. The clonal spread of imipenem-resistant *Acinetobacter* spp. accompanied by the wide dissemination of the OXA-23 carbapenemase has been noted in China^[92]. The first CRAB outbreak was reported in America in 1991, followed by global CRAB dissemination^[93]. Most outbreaks caused by CRAB have occurred in ICU settings^[29,94] throughout many countries. An outbreak caused by pandrug-resistant *A. baumannii* (PDRAB) was also reported in a pediatric ICU in a Taiwanese hospital^[95].

COMPARATIVE GENOMICS

In recent years, whole-genome sequencing and comparative genomics have been performed to elucidate the genetic basis of *A. baumannii* resistance, especially regarding the extent of variability and the acquisition and transfer of resistance determinants among different strains. The *A. baumannii* strain AYE, an endemic strain in France, exhibits an 86-kb resistance island in which 45 resistance genes are clustered^[96]. Sequence similarities and phylogenetic analyses confirm that most of the resistance genes

found in the *A. baumannii* AYE strain have been acquired from bacteria of the genera *Pseudomonas*, *Salmonella*, or *Escherichia*. Using pyrosequencing and transposon mutagenesis, the assembled genome of *A. baumannii* ATCC 17978 has been shown to consist of 3976746 base pairs (bp) and 3830 open reading frames (ORFs), a significant fraction (17.2%) of which are located in 28 putative alien islands^[97]. A remarkable number of the islands contain genes implicated in virulence. *A. baumannii* ACICU has a single chromosome of 3904116 bp and two plasmids, pACICU1 and pACICU2, of 28279 and 64366 bp, respectively^[98]. As many as 36 putative alien islands (pAs), 15 of which encode genes related to drug resistance, have been detected in the ACICU genome. One investigation involving MDRAB strains from hospitals of 10 European countries showed that AbaR3 is the original structure from which the AbaRs, the genomic islands containing many resistance genes, have been derived in European clone I, thus providing the strains of this lineage with a selective advantage^[99]. All of these findings indicate that the genome of *A. baumannii* has acquired a large amount of foreign DNA, which has an important role in pathogenesis and antimicrobial resistance.

Currently, the whole-genome sequencing of the widely spread MDRAB strain MDR-ZJ06^[100], an MDR-TJ^[101] strain in China; and two other multidrug-resistant strains (TCDC-AB0715, harboring both *bla*_{OXA-23} and *bla*_{OXA-66}^[102], and TYTH-1^[103] from Taiwan) has been completed. A comparative genomics analysis has revealed a common strain lineage between the Taiwanese strains (TYTH-1 and TCDCAB0715) and the Chinese strains (MDR-TJ and MDR-ZJ06)^[104]. Phylogenetic studies and GC profiles showed that the genome of TYTH-1 was the closest to the genome of MDR-ZJ06, which implies that the dissemination of *bla*_{OXA-23}-carrying CRAB in Taiwan may have been mediated by the transfer of people between Taiwan and China.

Adams *et al*^[105] found that the entire multidrug-resistance phenotype of *A. baumannii* can be explained by the acquisition of discrete resistance determinants distributed throughout the genome. A comparison of closely related multidrug-resistant and drug-susceptible isolates suggests that drug efflux may contribute less to the resistance to certain classes of antibiotics than inactivation of enzymes. A resistance island with a variable composition of resistance determinants interspersed with transposons, integrons, and other mobile genetic elements is a significant contributor to the multidrug-resistant phenotype. A whole-genome sequencing analysis of six closely related clinical isolates of *A. baumannii*, including four from one hospital, revealed an extensive divergence of the resistance genotype that correlated with the observed differences in antimicrobial susceptibility^[106]. Resistance genes associated with insertion sequences, plasmids, and a chromosomal resistance gene island all showed certain degrees of variability. The dynamic resistance gene pool suggests the rapid evolution of drug resistance in *A. baumannii*. The whole-genome sequencing of three dominant *A. baumannii* strains in an outbreak concluded that

much of their diversification was mediated by homologous recombination across 20% of their genomes^[107]. The differences in genomic contents among different *Acinetobacter* spp. are partly shaped by their distinct ecological niches^[108]. This notion is further supported by the variable presence of some genes encoding transcription factors and transporters among clinical isolates and their environmental *Acinetobacter* spp.^[105].

RESISTANCE MECHANISMS

Overview

Currently, certain strains of *A. baumannii* is highly resistant to most antibiotics available in clinical practice. A number of resistance mechanisms to many classes of antibiotics are known to exist in *A. baumannii*, including β -lactamases, multidrug efflux pumps, aminoglycoside-modifying enzymes, permeability defects, and the alteration of target sites^[109-111]. Most of these resistance mechanisms can target different classes of antibiotics. However, several different mechanisms can work together to contribute to the resistance to a single class of antibiotics. For example, the resistance mechanisms in CRAB are diverse^[112]. In addition to β -lactamases with carbapenem-hydrolyzing activity as a major carbapenem resistance mechanism, which include carbapenem-hydrolyzing class D β -lactamases (CHDLs) and metallo- β -lactamases (MBLs), porins such as CarO^[66] and penicillin-binding protein modifications might also be involved in carbapenem resistance^[113]. The spread of multidrug-resistance determinants in *A. baumannii* is mostly through plasmid conjugation, transposon acquisition or integron mobilization to gain clusters of genes encoding resistance to several antibiotic families^[110]. Furthermore, the functional insertion sequences are important in amplifying antimicrobial resistance and gene plasticity^[114-118]. Table 1 shows the various antimicrobial resistance mechanisms of *A. baumannii*. The details are further discussed below.

β -lactamase

Inactivation of β -lactams constitutes an important part of multidrug resistance in *A. baumannii*, especially for β -lactam antibiotic resistance. All four Ambler classes of β -lactamases (*i.e.*, classes A, B, C and D) can be identified in this organism^[66]. Although a wide range of class A β -lactamases, including those of temoneira (TEM)^[119-121], sulfhydryl variable (SHV)^[122], cefotaxime hydrolyzing capabilities (CTX-M)^[123,124], guiana extended-spectrum (GES)^[115,125], self-transferable plasmid from *E. coli* (SCO)^[126], *Pseudomonas* extended resistant (PER)^[127-130], vietnam extended-spectrum β -lactamase (VEB)^[96,131-133], carbenicillin hydrolyzing β -lactamase (CARB)^[134,135] and *K. pneumoniae* carbapenemase (KPC)^[136], have been reported in *A. baumannii* (Table 1), they are generally regarded to play a minor role in its resistance phenotype, especially in carbapenem resistance. Some of these enzymes are narrow-spectrum β -lactamases, *e.g.*, TEM-1^[119-121], SCO-1^[126] and CARB-4^[135]; however, a number of these enzymes are still responsible for the hydrolysis of

extended-spectrum β -lactams (ESBL). PER-1 was the first ESBL enzyme identified in *A. baumannii*^[137], whereas TEM-92 and CARB-10 were the first reported TEM-type^[120] and CARB-type^[134] ESBLs, respectively. Later, the chromosomally encoded ESBLs SHV-5^[122], PER-2^[132] and PER-7^[129,130] were also described. *A. baumannii* strains carrying the extended spectrum VEB-1 enzyme were first reported in an outbreak in France^[131]. GES-11, an integron-associated GES variant, can even confer reduced susceptibility to carbapenems^[115,125]. In addition, CTX-M enzymes are transmitted by integrons or plasmids, indicating the potential dissemination in outbreaks between different strains^[123,124]. Finally, KPC-10 was the first KPC β -lactamase to be identified^[136].

Class B β -lactamases can confer resistance to the majority of β -lactams because of their broad range, potent carbapenemase activity and resistance to inhibitors^[138]. Although MBLs are not the predominant carbapenemases in *A. baumannii*, verona integron-encoded metallo- β -lactamase (VIM), imipenemase (IMP) and seoul imipenemase (SIM) MBLs have been found contribute to the high-level resistance to carbapenems. The first VIM enzyme was described by Yum in 2002^[139]. Thereafter, several other VIM variants, including VIM-1^[140-142], VIM-3^[143], VIM-4^[141,142], and VIM-11^[143], were identified in *A. baumannii*. IMP enzymes have also been reported in several Gram-negative bacteria worldwide, including *A. baumannii*. At least nine variants of IMP enzymes have been identified in *A. baumannii*: IMP-1^[144], IMP-2^[145], IMP-4^[146,147], IMP-5^[148], IMP-6^[149], IMP-8^[143], IMP-11^[150], IMP-19^[150] and IMP-24^[143]. SIM-1 is the only SIM enzyme that has been reported in *A. baumannii*^[151]. More recently, NDM (new Delhi metallo- β -lactamase)-1^[152-154] and NDM-2^[155] were observed in *A. baumannii*. *bla*_{NDM-1} is integrated in the chromosome within a new transposon structure with two copies of the insertion sequence *ISA-ba125* in one clinical strain of *A. baumannii*. Such variability of the genetic environment of *bla*_{NDM-1} likely facilitates its rapid dissemination^[153].

The nucleotide sequence of the chromosomal cephalosporinase gene, which encodes an AmpC β -lactamase, in *A. baumannii* was first characterized in a clinical isolate from Spain in 2000^[156]. Different isolates of *A. baumannii* have been shown to have almost identical AmpC sequences (no more than two amino-acid substitutions)^[157]. A phylogenetic analysis showed that *Acinetobacter ampC* genes are descended from a common ancestor and are more closely related to each other than the *ampC* genes found in other species of bacteria^[158]. The class C chromosomal β -lactamase AmpC in *A. baumannii* has a typical cephalosporinase substrate profile^[156]. The presence of AmpC β -lactamase plays an important role in β -lactam resistance in *A. baumannii*, and in fact, a high percentage of drug-resistant *A. baumannii* possess *bla*_{ampC}^[119]. In a study of 23 MDRAB clinical isolates from five proximal hospitals in Taiwan, all isolates had AmpC-type *bla*^[77]. The presence of an insertion sequence with a strong promoter upstream of *ampC* in *A. baumannii* clinical isolates has the potential to overexpress AmpC, resulting in high-

Table 1 Antimicrobial resistance mechanisms in *Acinetobacter baumannii*

Resistance mechanism	Class/family	Protein	Ref.
β-lactamases	Class A	TEM-1	[105,119,121]
		TEM-92	[120]
		SHV-5	[122]
		CTX-M-2	[123]
		CTX-M-15	[124]
		GES-11	[115,125]
		GES-12	[115]
		GES-14	[115]
		SCO-1	[126]
		PER-1	[127,128]
		PER-2	[132]
		PER-7	[129,130]
		VEB-1	[96,105,131-133]
		CARB-4	[135]
		CARB-10	[134]
		KPC-10	[136]
	Class B	VIM-1	[140-142]
		VIM-2	[139,143]
		VIM-3	[143]
		VIM-4	[141,142]
		VIM-11	[143]
		IMP-1	[144]
		IMP-2	[145]
		IMP-4	[146,147]
		IMP-5	[148]
		IMP-6	[149]
		IMP-8	[143]
		IMP-11	[150]
		IMP-19	[150]
		IMP-24	[143]
		SIM-1	[151]
		NDM-1	[152-154]
		NDM-2	[155]
	Class C	AmpC	[156-160]
	Class D		
	Narrow-spectrum		
		OXA-3	[163]
		OXA-20	[164]
	group	OXA-10	[387]
	CHDLs		
	OXA-23	OXA-23	[66,92,105,147,167-183]
		OXA-133	[185]
	OXA-24	OXA-40/24	[197,201,204]
		OXA-40	[188,200, 202,203]
	group	OXA-72	[92,205,206]
		OXA-25, OXA-26, OXA-27	[198]
	OXA-51	OXA-51	[105,187-190]
		OXA64, OXA-65, OXA-66, OXA-68, OXA-70, OXA-71	[191]
		OXA-69, OXA-75, OXA-76, OXA-77	[186]
		OXA-79, OXA80, OXA-104, OXA106~OXA-112	[194]
		OXA-82, OXA-83, OXA-84	[192,194]
		OXA-86, OXA-87	[193]

		OXA-88, OXA-91, OXA-93, OXA-94, OXA-95, OXA-96	[147]
		OXA-92	[195]
		OXA-113	[122]
	OXA-58 group	OXA-58	[116,118,207,210,211, 215,219]
		OXA-96	[147]
		OXA-97	[220]
	Novel groups	OXA-143	[196]
		OXA-182	[221]
		OXA-235	[222]
Efflux pumps	RND	AdeABC	[235,238]
		AdeFGH	[243]
		AdelJK	[244]
	MFS	TetA	[248]
		CmlA	[225]
		MdfA	[233]
		CraA	[249]
		AmvA	[250]
	MATE	AbeM	[251]
	SMR	AbeS	[252]
AME	AAC	AAC3 (aacC1, aacC2)	[256]
		AAC (6') (<i>aacA4</i>)	[17,253,257-259,261]
	ANT	ANT (2'') (<i>aadB</i>)	[256]
		ANT (3'') (<i>aadA1</i>)	[17,253,261]
	APH	APH (3') (<i>aphA1</i>)	[255]
		APH (3'')	[253]
Permeability defects		CarO	[263-267]
		47-kDa OMP	[91]
		44-kDa OMP	[91]
		37-kDa OMP	[91]
		33-36-kDa OMP	[269]
		22-33-kDa OMP	[268]
		43-kDa OMP	[271]
		Lipopolysaccharide	[64]
		OmpA	[274]
Alteration of target sites	Change of PBP	PBP2	[276]
	DNA gyrase	GyrA/ParC	[237]
	Ribosomal protection	TetM	[280]
	Dihydrofolate reductase	Dfr or Dhfr	[17,281]
		FolA	[281]
	16S rRNA methylation	ArmA	[253,258,282-286]

TEM: Temoneira; SHV: Sulfhydryl variable; CTX-M: Cefotaxime hydrolyzing capabilities; GES: Guiana extended-spectrum; SCO: Self-transferable plasmid from *E. coli*; PER: *Pseudomonas* extended resistant; VEB: Vietnam extended-spectrum β-lactamase; CARB: Carbenicillin hydrolyzing β-lactamase; KPC: *K. pneumoniae* carbapenemase; VIM: Verona integron-encoded metallo-β-lactamase; IMP: Imipenemase; SIM: Seoul imipenemase; NDM: New Delhi metallo-β-lactamase; AmpC: Ampicillin class C β-lactamase; CHDL: Carbapenem-hydrolyzing class D β-lactamase; OXA: Oxacillinase; RND: Resistance-nodulation-division; MFS: Major facilitator superfamily; MATE: Multidrug and toxic compound extrusion; SMR: Small multidrug resistance; Ade: *A. baumannii* multidrug-resistant efflux pump; TetA: Tetracycline resistant *Acinetobacter*; CmlA: Chloramphenicol resistance *Acinetobacter*; MdfA: Multidrug facilitator; CraA: Chloramphenicol resistance *Acinetobacter*; AmvA: *A. baumannii* Methyl Viologen and antimicrobial resistance protein; AbeM: *A. baumannii* efflux pump of MATE family; AbeS: *A. baumannii* efflux pump of SMR family; AME: Aminoglycoside-modifying enzyme; AAC: Aminoglycoside acetyltransferases; ANT: Aminoglycoside adenylyltransferases; APH: Aminoglycoside phosphotransferases; CarO: Carbapenem-associated outer membrane protein; OMP: Outer membrane protein; PBP: Penicillin binding protein; GyrA/ParC: DNA Gyrase/partitioning of the nucleoid partition; Dhfr: Dihydrofolate reductase; FolA: Folate; ArmA: Armillaria mellea.

level ceftazidime resistance^[159,160]. IS*Aba1*-like sequences have been identified immediately upstream of the *bla_{ampC}* gene in ceftazidime-resistant *A. baumannii* isolates but have been shown to be absent in ceftazidime-susceptible *A. baumannii* isolates^[157].

Class D β -lactamases were designated OXAs in reference to their preferred substrate oxacillin^[161]. Some OXAs are also able to hydrolyze extended-spectrum cephalosporins, and some can even inactivate carbapenems by acting as carbapenemases^[66]. At least 121 different variants of class D β -lactamases have been identified at the protein level, and in contrast to other class D β -lactamases, 45 of these variants exhibit carbapenem-hydrolyzing activities^[162]. The *bla_{oxa}* genes can be located either on a chromosome or a plasmid and can sometimes be found in integrons^[163,164]. Among the four classes of β -lactamases, MBLs and CHDLs are the two main groups of carbapenemases in *A. baumannii*, the latter of which is responsible for the most common type of carbapenem resistance *via* enzymatic degradation^[165]. Currently, nine major subgroups of OXA carbapenemases have been identified based on amino acid homologies^[166]. Four subgroups of OXA with carbapenemase activity, including the OXA-23, OXA-40/24, OXA-51 and OXA-58 clusters, are prevalent in *A. baumannii*^[162,166].

New OXA-type carbapenemases have been frequently discovered since the first clinical isolate of *A. baumannii* with OXA-23 was characterized^[66]. The *bla_{OXA-23}* carbapenemase gene has also been disseminated worldwide^[167]. The countries that have reported *A. baumannii* with OXA-23 carbapenemase include France^[168-170], Germany^[171], Bulgaria^[172], Romania^[173], the United States^[105], Colombia^[174], Brazil^[175], Australia^[176], Taiwan^[177,178], China^[92,179], Korea^[180], Singapore^[147,181], Italy^[182] and Spain^[183]. *A. radioresistens* has been proposed as a silent source of *bla_{OXA-23}* for *A. baumannii*^[184], and a novel variant, named *bla_{OXA-133}*, has been reported by the Asia-Pacific SENTRY surveillance program^[185].

OXA-51/69-like β -lactamases are intrinsic chromosomal enzymes in *A. baumannii*^[166,186] that emerged as a new subgroup of carbapenemases in MDRAB in 2004^[187] and that show increased carbapenemase activity when IS*Aba1* is upstream of the promoter region^[188,189]. However, drug export by an efflux pump might be more important in some clinical isolates^[190]. A comparative genomics study by Adams *et al.*^[105] showed that the studied *A. baumannii* strains, including wild-type strains and clinical isolates of MDRAB, all possessed genes belonging to the OXA-51 group. The recently identified OXA-51 group of β -lactamases comprises a novel cluster among the OXA-type carbapenemases, and the cluster includes many variant oxacillinases that have been reported in several studies, including those by Heriter in 2005^[186], Brown in 2005^[191], Turton in 2006^[192], Vahaboglu in 2006^[193], Koh in 2007^[147], Evans in 2007^[194], Naas in 2007^[122], Tsakris in 2007^[195] and Higgins in 2009^[196]. The CHDLs that have been found are listed in Table 1.

The OXA-40/OXA-24 CHDL group is made up of OXA-25, OXA-26, OXA-40, and OXA-72 (an original

sequencing error occurred in sequencing OXA-24; it is now known as OXA-40)^[166]. These enzymes only differ by a few amino acid substitutions. OXA-40/OXA-24 was originally identified as chromosomally encoded in a carbapenem-resistant *A. baumannii* isolate recovered from Spain^[197]. OXA-25, OXA-26, and OXA-27 were later characterized to be associated with carbapenem resistance in clinical isolates of *A. baumannii* from Spain, Belgium and Singapore^[198]. Thereafter, the OXA-40/OXA-24 gene in *A. baumannii* was reported in several areas^[199], including Spain^[188,200,201], Portugal^[202] and the United States^[203]. The plasmid-mediated *bla_{OXA-24}* gene was noted in the isolates from an outbreak in Spain^[204]. Additionally, OXA-72 has been identified in *A. baumannii* isolates from Taiwan^[205], China^[92] and Croatia^[206].

OXA-58 was first identified from an isolate of MDRAB in France^[207]. The *bla_{OXA-58}* gene was found to be plasmid borne. Many OXA-58-producing *A. baumannii* isolates were reported worldwide in subsequent years, including isolates in Europe^[208-211], Argentina^[208], Kuwait^[208], the United Kingdom^[208], Australia^[212], Taiwan^[116], the United States^[213,214] and China^[215]. A number of outbreaks have also been reported in many countries, including Italy^[216], Belgium^[79], France^[217], Turkey^[193], Greece^[218,219], and the United States^[214]. OXA58 can lead to high-level carbapenem resistance in *A. baumannii* *via* the upstream IS1008 insertion^[116] or the presence of the IS*Aba25*-IS*Aba3*-like hybrid promoter^[118]. OXA-97 is a point mutation variant of OXA-58 that shares the same hydrolytic properties and has been recently identified in *A. baumannii* isolates from Tunisia^[220]. Another point mutation derivative is OXA-96, which was identified in *A. baumannii* from Singapore^[147].

In 2009, a novel CHDL, OXA-143, was identified that shares 88% amino acid identity with OXA-40, 63% identity with OXA-23, and 52% identity with OXA-58^[196]. Another novel oxacillinase, OXA-182, was identified in imipenem-nonsusceptible *Acinetobacter* isolates in Korea^[221] and showed 93% identity with OXA-143 and 89% identity with OXA-40 based on amino acid sequence alignment. OXA-235, and the amino acid variants OXA-236 and OXA-237, were identified from *A. baumannii* isolates from the United States and Mexico^[222]. The deduced amino acid sequences shared an 85% identity with OXA-134, 54 to 57% identities with the acquired OXA-23, OXA-24, OXA-58, and OXA-143, and a 56% identity with the intrinsic OXA-51. Thus, OXA-235, OXA-236 and OXA-237 represent a novel subclass of OXAs. The expression of OXA-235 in *A. baumannii* leads to reduced carbapenem susceptibility, while the cephalosporin minimal inhibition concentrations (MICs) are unaffected.

Multidrug efflux pumps

While multidrug-resistant efflux pumps have been shown to have roles in bacterial pathogenicity^[223], the contribution of efflux pumps to bacterial multidrug resistance is often reported^[224,225]. Efflux-based mechanisms are responsible for resistance against many different classes of antibiotics, including tigecycline resistance^[226,227] or imipenem resistance^[190] in *A. baumannii*. Furthermore, the

linear relationship between the log-transformed expression values of the AdeABC efflux pump genes and the log-transformed MIC values is statistically significant, indicating that overexpression of the AdeABC efflux pump is a prevalent mechanism for decreased susceptibility to tigecycline^[228]. The importance of efflux pumps in multidrug resistance in *A. baumannii* is supported by the fact that the presence of efflux pump inhibitors, such as 1-(1-naphthylmethyl)-piperazine^[229,230], phenyl-arginine- β -naphthylamide^[231,232], or carbonyl cyanide 3-chlorophenyl-hydrazone^[232], can reverse the resistance pattern.

Four categories of efflux pumps, including the resistance-nodulation-division (RND) superfamily, the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family and the small multidrug resistance (SMR) family transporters, have been reported to be related to antimicrobial resistance in *A. baumannii*^[233,234]. Of these different pumps, the RND and MFS transporters are mentioned most often. AdeABC, a RND-type efflux pump with a three-component structure, is not only associated with aminoglycoside resistance^[235] but is also associated with decreasing susceptibility to several antimicrobials, including tigecycline^[226]. Differences in the expression of *adeABC* were shown to contribute to both inter- and intra-clone variation in tigecycline MICs in a study of *A. baumannii* epidemic clones^[236]. Both the increase in tigecycline resistance during therapy^[236] and the decrease in susceptibility to non-fluoroquinolone antibiotics during an outbreak^[237] are mediated by the up-regulation of AdeABC in *A. baumannii*. The AdeABC pump in wild *A. baumannii* is cryptic due to stringent control by the AdeRS two-component system^[238]. Point mutations in AdeS and AdeR or a truncation of AdeS due to an IS*Aba1* insertion may be related to the overexpression of AdeABC, which leads to multidrug resistance^[238,239]. However, the existence of tigecycline-nonsusceptible and *adeB*-overexpressing *A. baumannii* clinical isolates without known *adeRS* mutations^[240] and the low expression of *adeABC* in a clinical strain of *A. baumannii* with the IS*Aba1* insertion in the *adeRS* operon^[239] suggest that the regulation of *adeABC* gene expression is complex. Additionally, the cell density-dependent expression of *adeB* suggests the presence of global regulatory mechanisms for the expression of this gene in *A. baumannii*^[241]. BaeSR, which functions as an envelope stress response system to external stimuli, is proposed to influence the transcription of *adeAB* and thus tigecycline susceptibility in *A. baumannii* by functioning as a regulator of global transcription^[242].

In addition to the AdeABC efflux pump, the inactivation of other RND-type efflux pumps, including AdeFGH^[243] and AdeIJK^[232,244,245], demonstrates their contribution to multidrug resistance in *A. baumannii*. The AdeABC and AdeIJK efflux systems can contribute synergistically to tigecycline resistance^[244]. An open reading frame encoding a LysR-type transcriptional regulator, named *adeL*, is located upstream of the *adeFGH* operon and is responsible for the overexpression of AdeFGH^[243], whereas the expression of AdeIJK in *Acinetobacter bau-*

mannii is regulated by AdeN, a TetR-Type regulator^[246]. Although the RND efflux pump AdeDE was initially identified in *Acinetobacter* genomic group 3^[247], *adeE* was later found to coexist with *adeB* in some clinical isolates of *A. baumannii*^[245].

A number of MFS efflux pumps, including TetA^[248], CmlA^[225], MdfA^[233], CraA^[249] and AmvA^[250], that mediate resistance to different types of antibiotics in *A. baumannii* have been characterized. AbeM, a H-coupled pump that belongs to the MATE family^[251], was reported to be present in the clinical isolates of *A. baumannii* in several studies^[77,232,245] and to confer resistance to fluoroquinolones or imipenem in *A. baumannii*. *A. baumannii* with a mutant AbeS SMR pump exhibits erythromycin and chloramphenicol resistance^[252].

Aminoglycoside-modifying enzymes

Aminoglycoside-modifying enzymes (AMEs) are the principal mode of resistance to aminoglycosides. This resistance is primarily mediated by three classes of enzymes, including acetyltransferases, adenylyltransferases and phosphotransferases, that typically reside on transposable elements; these enzymes chemically modify aminoglycosides^[253]. The coding genes for these enzymes can be transferred among different bacterial types through plasmids, transposons, integrons, and natural transformation or transduction^[254]. A phenotypic analysis of aminoglycoside resistance profiles indicated that many isolates could produce a combination of aminoglycoside-modifying enzymes^[255,256]. The co-carrying of four AME genes, including a novel AME gene *aac(6')-Ib*, was reported in a PDRAB strain from China^[257]. The identification of MDRAB isolates harboring genes for the *bla*_{OXA-23}-like genes, AME (*aac(6')-Ib*) and the 16S rRNA methylase (*armA*) implicates AMEs in multidrug resistance^[258].

Different types of AMEs have been reported in *A. baumannii*. Amikacin resistance has been reported to be associated with a gene encoding APH(3')-VI phosphotransferase^[255]. Furthermore, AME *aac(6')-Iad* plays an important role in amikacin resistance in *Acinetobacter spp.* in Japan^[259]. Of the 106 MDRAB isolates identified in one study, 95% possessed at least one type of AME, including *aacA4*, *aacC1*, *aacC2*, *aadB*, *aadA1*, *aphA1* and *aphA6*^[256]. In another study in Greece, all of the collected *A. baumannii* strains contained AMEs, which were either *aac(6')-Ib* or *aac(6')-Ib*^[260]. Class I integrons containing the gene cassettes *aacA4-catB8-aadA1*, *dhfrXII-orfF-aadA2*, or *aacC1-orfP-orfP-orfQ-aadA1* have been proposed to be associated with the horizontal transfer of diversified aminoglycoside-resistant genes among clinical isolates of *A. baumannii*^[17,256,261].

Permeability defects

Porins, which perform multiple functions in membranes, are proteins that can form channels to allow the transport of molecules across lipid bilayer membranes^[233]. These outer membrane proteins not only influence the virulence of *A. baumannii*, e.g., through Omp38-induced epithelial cell apoptosis^[59], biofilm formation related to OmpA^[262],

OmpA-dependent host cell death^[263], and attenuated virulence by the decreased expression of genes encoding CarO- and OprD-like proteins^[263], but also play a significant role in the mechanisms of resistance. For example, the loss of a 29 kDa outer-membrane protein, which was later shown to be CarO, contributes to imipenem resistance^[263-267]. Several other studies have also identified a number of OMPs involved in the carbapenem resistance of *A. baumannii*. A reduction in the expression of two porins of 22 and 33 kDa was involved in the carbapenem resistance of *A. baumannii* strains in an outbreak in Spain^[268]. In one study, CRAB isolates found in New York had reduced expression of the 47-, 44-, and 37-kDa outer-membrane proteins^[91], while in other studies, a 33- to 36-kDa OMP was also shown to be associated with carbapenem resistance in *A. baumannii*^[269,270]. A 43-kDa OMP, belonging to the OprD family, has been identified as a basic amino acid and imipenem porin through electrophoresis and MALDI-MS analyses^[271].

In the presence of OXA carbapenemases, including OXA-51-like or OXA-23-like enzymes, the loss of the 29-kDa outer-membrane protein is associated with a higher imipenem MIC in *A. baumannii*^[272,273]. Moreover, a novel insertion sequence, IS*Aba10*, inserted into IS*Aba1* adjacent to the *bla*_{OXA-23} gene, can disrupt the outer-membrane protein gene *carO* in *A. baumannii*^[180]. The loss of lipopolysaccharide (LPS) from the outer membrane, resulting in a decrease in membrane integrity, occurred in a colistin-resistant clinical isolate of *A. baumannii* in Australia^[64]. Disruption of the *ompA* gene can lead to decreases in the MICs of chloramphenicol, aztreonam, and nalidixic acid^[274].

Alteration of target sites

Changes in penicillin-binding proteins (PBPs), mutations of DNA gyrase, ribosomal protection by the TetM protein and the involvement of dihydrofolate reductase in trimethoprim resistance all occur *via* mechanisms that alter the target sites for antibiotics^[275]. Imipenem resistance has been associated with the overexpression of certain PBPs with a low affinity for imipenem in the absence of other known resistance mechanisms^[276]. While an insertion sequence disrupting the gene encoding PBP6b has been identified in an endemic carbapenem-resistant clone, its role must be further evaluated^[277]. Furthermore, mutations in DNA gyrase gene *gyrA* and *parC*, which encode topoisomerase IV, have been reported in an *A. baumannii* outbreak investigation^[237]. The *gyrA* mutation at Ser-83 was shown to be associated with quinolone resistance in epidemiologically unrelated isolates of *A. baumannii*^[278]. While *tetA* and *tetB* genes are well recognized for their role in tetracycline resistance in *A. baumannii* through efflux pumps^[225,279], *tetM* is proposed to be another resistance mechanism that acts through ribosomal protection^[280]. Trimethoprim resistance through dihydrofolate reductase in *A. baumannii* is similar to that of other bacteria. Plasmids containing *folA* genes and integrons harboring *dhfr* or *dhfr* genes in *A. baumannii* have been found^[117,279,281]. Recently, the coexistence of the 16S rRNA

methylase *armA* gene and genes encoding OXA carbapenemases were reported in many countries, including China^[282], South Korea^[253,283], India^[284], Italy^[285], Japan^[286], and Yemen^[258], indicating the contribution of the *armA* gene to the multidrug resistance of MDRAB.

Roles of integrons

The horizontal transfer of resistance genes is a successful mechanism for the transmission and dissemination of multiple drug resistance determinants among bacterial pathogens^[287]. Integrons, which are located on either bacterial chromosomes or plasmids, are assembly platforms that incorporate exogenous ORFs by site-specific recombination and convert them to functional genes by ensuring their correct expression^[288]. Integrons share common features: a gene encoding an integrase, a specific recombination site that is recognized by the integrase and into which the cassettes are inserted, and a promoter that directs the transcription of the cassette-encoded genes. Currently, there are four classes of integrons, and class 1 integrons are the most common in bacteria^[289].

The role of integrons in the development of multidrug resistance relies on their unique capacity to cluster and express drug resistance genes^[287]. Many studies regarding integrons harboring different types of resistance genes have been reported worldwide in recent decades. Class I integrons were detected in 52.8% of *A. baumannii* strains in the Nanjing area of China in 2007^[290], whereas an epidemic, class 1 integron-carrying MDRAB clone was found to be widespread in Taiwan in the same year^[291]. Four different integron structures were detected in 84% of all collected isolates of *A. baumannii* in a Spanish study^[255]. However, while no clear antibiogram differences could be associated with the presence or absence of integron structures in the Spanish study, other reports have suggested that integrons play a major role in multidrug resistance in *A. baumannii*^[261,291,292]. Additionally, epidemic strains of *A. baumannii* have been found to contain significantly more integrons than non-epidemic strains^[293]. Therefore, integrons are regarded as useful markers for epidemic strains of *A. baumannii*, and their typing can provide valuable information for epidemiological studies^[294,295].

A study performed in Italy found that 44% of the epidemiologically unrelated *A. baumannii* isolates collected over an 11-year period were integron-positive^[296]. Most integron-positive strains carried the same array of cassettes, despite their notable genetic diversity that was identified through a ribotyping analysis, implying that horizontal transfer of the entire integron structure or an ancient acquisition occurred. Additionally, while the same integron can be present in unrelated strains^[17], related strains can also have different integrons^[297].

Although different relationships exist among different classes of antibiotics and integrons^[298,299], most studies have emphasized the association of integrons with cassette genes and aminoglycoside resistance^[261]. The diversity of the genes encoding AMEs and their association with class 1 integrons was observed in a study involving

three pan-European clones of *A. baumannii*^[256]. Six different class 1 integron variable regions were detected in 74% of the collected strains. Furthermore, Huang *et al*^[291] collected 283 MDRAB isolates from three medical centers in Taiwan from 1996 to 2004 and found seven types of gene cassettes, most of which contained AMEs, including *aacA4*, *aacC1*, *aac(6)-II*, *aadA1*, *aadA2*, *aadA4* and *aadDA1*.

Variable CHDL genes, including *bla_{OXA-3}*^[292], *bla_{OXA-10}*^[96,290], *bla_{OXA-20}*^[19,292,296], *bla_{OXA-21}*^[297], and *bla_{OXA-37}*, have been reported in integrons^[164,297]. Integron-associated imipenem resistance in *A. baumannii* has also been documented^[300]. Genes encoding carbapenemases, such as MBLs *bla_{VIM}*, *bla_{IMP}* and *bla_{SIM}*, have been found in integrons. *bla_{VIM-1}*-carrying integrons^[140] and *bla_{VIM-2}*-carrying integrons^[139] have been noted in Greece and Korea, respectively. In Taiwan, integron-mediated gene spreading has been demonstrated hospitals^[301], especially in a unit with high antibiotic selective pressure^[302]. *bla_{VIM-11}*-carrying integrons have also been identified in MDRAB isolates, and this MBL gene has been postulated to spread among *Pseudomonas aeruginosa* and *A. baumannii* strains^[143,291]. Other reported MBLs include *bla_{IMP-1}*^[303], *bla_{IMP-2}*^[145], *bla_{IMP-4}*^[146,147], *bla_{IMP-5}*^[148], *bla_{IMP-8}*^[291] and *bla_{SIM-1}*^[151]. The genes for chloramphenicol resistance in the integrons of *A. baumannii* are *catB2*^[135], *catB3*^[146,147,151] and *catB8*^[294,304].

CLINICAL IMPACT OF ANTIMICROBIAL RESISTANCE

The clinical impact of *A. baumannii* infections has been a matter of debate^[2]. A high mortality rate in immunocompromised hosts with *A. baumannii* infections had been attributed to the patients' underlying diseases rather than to the infections. One Spanish study concluded that there were no differences in mortality among patients with ventilator-associated pneumonia (VAP) caused by imipenem-resistant or imipenem-susceptible *A. baumannii* or by other pathogens^[305]. However, other related studies suggest that *A. baumannii* infection itself has a profound influence on high mortality or prolonged length of stay (LOS)^[306]. Falagas suggested that the mortality attributed to *A. baumannii* infections should no longer be a controversial issue^[307] based on six relevant case-control studies^[308-313].

Several previous surveillance^[314-317] studies have demonstrated that increasing antimicrobial resistance, especially multidrug resistance, has become a major issue in *A. baumannii* strains in recent years. Whether multidrug resistance is a risk factor for high mortality in *A. baumannii* infections has been a controversial issue. A few studies suggested that MDRAB-related pneumonia or bacteremia is a signal of disease severity and is not related to prolonged LOS or increased mortality^[318,319], but more recent studies have shown that MDRAB infections lead to higher mortality. The acquisition of MDRAB was shown to be an independent risk factor for mortality in a burn center in Singapore^[320]. A multicenter retrospective study in Taiwan

also showed that patients with CRAB infections have a higher mortality rate than those with carbapenem-susceptible *A. baumannii* infections^[321], which is consistent with the results of several previous studies^[309-311,313,322]. The high impact of imipenem resistance on the mortality rate of patients with *Acinetobacter* bacteremia is chiefly attributable to discordant antimicrobial therapy^[311]. Moreover, patients with MDRAB infections have increased hospital and ICU LOS compared to patients with susceptible *A. baumannii* infections and uninfected patients^[308]. A mini review of this issue indicated that blood stream infections and nosocomial ICU infections caused by carbapenem-resistant *Acinetobacter* spp. are associated with increased rates of mortality, whereas other types of infections have not clearly been shown to be associated with higher mortality rates but are associated with increased LOSs and hospital costs^[323].

STRATEGIES TO COMBAT THE DISSEMINATION OF ANTIMICROBIAL RESISTANCE

The development of new antibiotics against MDRAB and the implementation of infection control measures are regarded as two methods to aid in controlling the increasing problem of *A. baumannii* infections^[307]. When GlaxoSmithKline shared the challenges and difficulties in screening for new classes of antimicrobial agents over a 7-year period, the authors concluded that the pipeline of novel-mechanism antibacterials is still empty and will remain so for a considerable period^[324]. Therefore, the importance of following the Association of Professionals in Infection Control and Epidemiology's (APIC) 2010 guide to the elimination of MDRAB transmission in health care settings cannot be overemphasized^[325]. This guide includes MDRAB risk assessment and infection surveillance, strict adherence to hand hygiene protocols, implementation of standard and transmission-based precautions, environmental decontamination, outbreak recognition and control, and antibiotic stewardship.

Gastrointestinal or skin colonization of *A. baumannii* develops soon after the pathogen is first isolated from a clinical site^[326]. The finding of multidrug-resistant colonized strains compared with susceptible clinical strains without apparent relation to antibiotic use implies that a new onset of MDRAB colonization may not be identified without surveillance. Additionally, the increasing occurrence of multidrug-resistant strains among seriously ill patients emphasizes the importance of continued surveillance as a critical component of any program aimed at preventing and controlling antimicrobial resistance^[315]. Environmental contamination, airborne transmission, patient transfer, and cross-contamination are regarded as key factors in causing *A. baumannii* epidemics^[327], and clonal expansion has been shown to play a major role in the increase of MDRAB in hospitals^[328]. Therefore, barrier infection control measures are necessary to prevent the nosocomial spread of MDRAB^[326]. One outbreak

Table 2 Antimicrobial treatment for MDRAB infections

Regimen	Pathogen	Diseases	Outcome ¹	Comparator	Ref.
CST + RIF	XDRAB	HAP VAP BSI cIAI	The same in CR (mortality) Better in MR	CST	[367]
CST + RIF	CRAB	VAP	The same in CR + MR	CST	[366]
CST + IPM	XDRAB	BSI	Better in CR (mortality) + MR	CST	[369]
CST + SAM					
CST + others					
CST + SUL	MDRAB	VAP	The same in CR + MR	CST	[341]
TGC based	MDRAB	Pneumonia	Higher mortality	CST based	[349]
TGC based	MDRAB	HAIs	The same in mortality ² Better in MR	IPM + SAM	[352]
CT	MDRAB	Infections	The same in mortality	MT	[374]

¹"The same" means no significant difference between comparator groups, and "Better" means a significant difference exists between comparator groups;

²Has a statistically significant favorable outcome. MDRAB: Multidrug-resistant *A. baumannii*; CST: Colistin; IPM: Imipenem; RIF: Rifampicin; SUL: Sulbactam; SAM: Ampicillin/sulbactam; TGC: Tigecycline; HAP: Hospital-associated pneumonia; VAP: Ventilator-associated pneumonia; BSI: Blood stream infection; cIAI: Complicated intra-abdominal infection; HAIs: Healthcare-associated infections; CR: Clinical response; MR: Microbiological response; CT: Combination therapy; MT: Monotherapy.

reported in an ICU in a Greek hospital ceased after the implementation of hygienic measures, complete cleaning and complete disinfection in the ICU^[329]. However, cross-infection with *A. baumannii* among patients still occurred, despite the implementation of stringent infection control measures, in a previously reported outbreak; thus, temporary closure of the surgical ward for disinfection was necessary to control the outbreak^[330].

Environmental contamination plays an important role in the transmission of MDRAB. One outbreak investigation found that the affected patients had a higher risk of harboring *A. baumannii* after blood transfusion, hydrotherapy or extended use of a respirator, which was possible through the contamination of healthcare personnel and the environment. Another *A. baumannii* outbreak investigation in a surgical ICU at a teaching hospital in Taiwan showed extensive amounts of environmental contamination, including the contamination of bed rails, bedside tables, sinks, ventilator and infusion pump surfaces, and water for nasogastric feeding and ventilator rinsing. Hence, intensified infection prevention control (IPC) measures are needed to terminate an outbreak. The IPC measures include: (1) implementation of enhanced contact isolation precautions; (2) active surveillance cultures; (3) daily environmental cleaning with detergents and phenolic agents; (4) an up-to-date education program for all healthcare workers; and (5) delivery of real-time feedback to healthcare workers regarding IPC compliance^[331], which has minimized the spread of colistin-resistant *A. baumannii*. Furthermore, the infection control bundle resulted in a significant reduction in the incidence of nosocomial *A. baumannii* in one burn unit and prevented further outbreaks of this organism, with an 88.8% decrease during the intervention period^[332].

Imipenem has been proven to be a strong inducer of multidrug resistance in *A. baumannii*^[333]. Many *A. baumannii* isolates exhibit imipenem resistance, which is strongly associated with the prior use of carbapenems^[334]. Because of the high mortality rate associated with *A. bauman-*

nii infection, strategies to slow down the emergence of MDRAB in clinical practice by optimizing antimicrobial therapy are necessary. Therefore, antimicrobial stewardship is mandatory in an infection prevention program to prevent the emergence and transmission of MDRAB in health care facilities^[325].

ANTIMICROBIAL THERAPY

Carbapenems, including imipenem or meropenem, have been regarded as effective antimicrobial agents to treat *A. baumannii* infections^[314,335]. With many studies reporting increasingly high rates of CRAB in clinical isolates^[75,76,90], other classes of antibiotics or combination therapies are urgently needed. Because the choices of antimicrobial treatment for MDRAB are severely limited by resistance, there are only a few effective options available, including polymyxins and tigecyclines^[336]. Furthermore, the appearance of PDRAB, which is resistant to all available antibiotics, including polymyxin, implies that more efforts should be devoted to investigating the treatment options for this superbug^[27]. Combination therapies with imipenem/sulbactam, colistin/rifampicin, colistin/sulbactam, colistin/tigecycline, colistin/imipenem or meropenem and colistin/teicoplanin have been studied and proposed as possible choices. The recently published reports on the treatment of MDRAB are summarized in Table 2.

Sulbactam

While ampicillin/sulbactam has been shown to be effective in treating blood stream infections caused by MDRAB^[337], a later meta-analysis revealed that sulbactam-based therapy is not superior to other therapeutic approaches, including colistin, cephalosporins, antipseudomonas penicillins, fluoroquinolones, minocycline/doxycycline, aminoglycosides, tigecycline, polymyxin, imipenem/cilastatin, and combination therapies^[338]. Although sulbactam-based therapy failed to prove its superiority to other regimens for the treatment of *A. baumannii* in-

fections, a case of skin and soft tissue infection caused by CRAB that was treated successfully with ampicillin/sulbactam and meropenem combination^[339] raises the possibility of ampicillin/sulbactam as a component of combination therapy against CRAB. The combination of ampicillin/sulbactam with a carbapenem for treating MDRAB bacteremia has been shown to be associated with a better outcome^[340], but such beneficial effects were not observed for MDRAB VAP^[341].

Tigecycline-based therapy

In 2004, tigecycline was reported to have a good *in vitro* bacteriostatic effect against *A. baumannii*, including strains resistant to imipenem^[342]. Another *in vitro* study using a time-kill assay demonstrated the potential role of tigecycline in the treatment of *A. baumannii* and proposed that doxycycline could be a suitable and cost-effective option in some instances^[343]. Tigecycline efficacy was shown to correlate well with the free concentration-time curve of MIC in a murine *Acinetobacter* spp. model^[344]. Additionally, several cases affiliated with severe infections by MDRAB were successfully treated with tigecycline in terms of their clinical and microbiological outcomes^[345].

With its increasing use, the limitations and adverse aspects of tigecycline in treating MDRAB infections have begun to be realized. Tigecycline was less effective than imipenem in the treatment of pneumonia caused by non-IRAB strains in a murine pneumonia model^[346]. In a study consisting of 34 patients with MDRAB infections, the mortality rate reached up to 41%. The authors found that the correlation of clinical and microbiological outcomes was poor and concluded that tigecycline had excellent *in vitro* activity against MDRAB, but its clinical efficacy was still uncertain^[346]. One of the possible causes for the discrepancy of treatment outcomes may be variable tigecycline MICs. MIC determination for tigecycline before treatment, with the broth dilution method being favored^[347], might increase clinical success^[345]. *A. baumannii* isolates with tigecycline MICs of ≥ 2 mg/L were associated with higher mortality rates; thus, treatment with β -lactams or carbapenems instead of with tigecycline is preferred^[348]. This notion was further supported in a matched cohort study in Taiwan that dealt with the effectiveness of tigecycline-based versus colistin-based therapy for the treatment of pneumonia caused by MDRAB^[349]. The excess mortality rate of 16.7% in the tigecycline-based group compared with the colistin-based group was mostly attributed to subjects with MIC > 2 μ g/mL.

In a meta-analysis of the efficacy and safety of tigecycline, clinical failure, superinfection and adverse events were more frequent with the use of tigecycline^[350]. The authors suggested that physicians should avoid tigecycline monotherapy for the treatment of severe infections caused by MDRAB and that they should use it as a last-resort antibiotic. There was no antagonism found when tigecycline was used with other antimicrobials possessing activities against Gram-negative bacteria^[351]. However, tigecycline-based therapy for MDRAB infections is not satisfactory. In a study of 266 patients with healthcare-

associated MDRAB infections, the mortality rate was not significantly different between those receiving tigecycline-based therapy and those receiving non-tigecycline therapy^[352].

While tigecycline has an expanded spectrum of antibacterial activity and a synergic effect with some classes of antibiotics, such as amikacin^[353], earlier studies have shown that tigecycline resistance in *A. baumannii* has emerged^[354] and is associated with multidrug efflux systems, especially overexpression of the *adeABC* pump^[226,227]. The increased expression of the *adeABC* operon can be found in clinical isolates of *A. baumannii* post-tigecycline therapy^[236,355]. High resistance rates and high MICs of tigecycline in multiple clones of MDRAB were noted in a medical center in Israel^[356]. This phenomenon led to concern regarding the use of tigecycline as one of the few treatment choices for infections caused by MDRAB.

Colistin-based therapy

Colistin has been described as a last resort for the treatment of MDRAB^[357], and this drug is often used in combination therapy. In a report on the clonal spread of MDRAB in eastern Taiwan, antibiotic susceptibility testing showed that 10.4%, 47.8% and 45.5% of MDRAB isolates were resistant to colistin, rifampicin, and tigecycline, respectively, implying that colistin was the only effective antimicrobial agent in that area for treating MDRAB^[358]. In addition to its intravenous injection for MDRAB infections, colistin can be given *via* intraventricular and intrathecal routes for meningitis^[359] and *via* nebulization for pneumonia^[360,361].

Unfortunately, colistin-resistant *A. baumannii* strains have been reported all over the world^[357] and are attributed to the loss of lipopolysaccharide^[64] or/and phosphoethanolamine modification of lipid A mediated by the PmrAB two-component system^[362,363]. Because colistin monotherapy is unable to curb the appearance of resistance, colistin-based combination therapy might be the optimal antimicrobial strategy. Colistin combined with different classes of antibiotics, including tigecycline, cefoperazone/sulbactam or piperacillin/tazobactam, revealed synergistic effects in some CRAB strains^[364]. Time-kill assays have also shown that colistin/meropenem, colistin/rifampicin, and colistin/minocycline are synergistic *in vitro* against XDRAB strains^[365]. The beneficial effects of colistin and rifampicin combination for patients with VAP caused by CRAB have been documented in terms of clinical and microbiological outcomes^[366]. However, another multi-center, randomized clinical trial concluded that 30-d mortality was not reduced by the addition of rifampicin to colistin in serious XDRAB infections^[367]. Additionally, such a regimen might be hindered by a high level of rifampicin resistance in *A. baumannii*^[368]. Treatment with combination therapy, including colistin/carbapenem and colistin/sulbactam, for XDRAB blood stream infections led to higher microbiological eradication and lower mortality rates in comparison with the colistin monotherapy group^[369]. The combination therapy

of colistin and tigecycline has also been proposed as a reasonable treatment of choice for XDRAB pneumonia, especially in the first 48 h, in a rat lung model^[370]. Interestingly, a significant synergy has been observed for the combination of colistin and teicoplanin against MDRAB *in vitro*^[371]. Telavancin, a similar lipoglycopeptide of teicoplanin, has been shown to be efficacious *in vivo* when used in colistin combination therapy in a *Galleria mellonella* model of *A. baumannii* infection^[372].

Other antimicrobial therapies

Doripenem, a novel broad-spectrum carbapenem, has displayed *in vitro* synergistic activity with tigecycline, colistin and amikacin against MDRAB strains with doripenem resistance^[373]. One recent prospective, observational Spanish study did not support an association of combination therapy with reduced mortality in MDRAB infections^[374]. Overall, the choice of combination therapy should take several key factors into consideration, including the antimicrobial resistance phenotype, resistance mechanisms, and MIC^[375].

FUTURE PERSPECTIVES

One of the difficulties encountered in understanding the antimicrobial resistance mechanisms of *A. baumannii* lies in the complexity of the involved genes. A DNA microarray, the Check-MDR CT102 microarray, has proven useful in detecting TEM, SHV and CTX-M extended-spectrum β -lactamases and KPC, OXA-48, VIM, IMP, and NDM-1 carbapenemases in some *Enterobacteriaceae* and glucose non-fermentative bacteria, including *A. baumannii*, with 100% sensitivity and specificity for most of the tested genes^[376]. The detection of plasmid-mediated cephalosporinases, including CMY-2-like, DHA, FOX, ACC-1, ACT/MIR and CMY-1-like/MOX, was also possible using this assay, suggesting that this DNA array is a powerful high-throughput tool for most common resistance gene identifications and provides a platform for epidemiological or infection-control studies^[377].

Bacteria develop resistance to new classes of antibiotics very quickly, and bacteria may even be resistant to new classes of antibiotics before they are introduced to clinical use^[378]. Hence, antimicrobial peptides (AMPs) may be another option due to the rare appearance of resistance to AMPs in addition to their antimicrobial and anti-inflammatory effects^[379]. AMPs are an important component of host defenses against invading pathogens^[380]. They are small, cationic and amphipathic peptides of variable length, sequence and structure. Thus far, more than 750 different AMPs have been identified in various organisms ranging from plants to animals, including humans, most of which exhibit broad-spectrum activity against a wide range of microorganisms by disrupting the plasma membrane and causing cell lysis. Three classes of AMPs, including defensins, cathelicidins, and histatins, have been found in humans^[379]. The cathelicidin family is currently limited to a single gene, CAMP. LL-37, which begins with

two leucine residues and consists of 37 amino acids, was the first mature peptide isolated from CAMP gene products^[381].

While only a few studies regarding the use of AMPs in *A. baumannii* have been reported, AMPs might be a potential therapeutic alternative to antibiotics. This hypothesis is supported by the conclusion reached from a study of an LPS-deficient, colistin-resistant *A. baumannii* strain, which showed reduced viability even at a low concentration of LL-37^[382]. The human antimicrobial peptide LL-37 and its fragments KS-30 and KR-20 have been shown to have significant antimicrobial activity against clinical isolates of MDRAB, of which the KS-30 fragment exhibits the highest bactericidal ability^[383]. Moreover, the prevention of biofilm formation *in vitro* by LL-37, KS-30 and KR-20 adds significance to their efficacy. We predict that AMPs, specifically LL-37, will be promising targets in future research on therapeutics against MDRAB infections.

Because marketing a new antimicrobial is extremely difficult and because bacteria quickly adapt to so-called magic bullets, understanding the interplay between a pathogen such as *A. baumannii* and its hosts may provide another possible solution in the war against bacteria. The microbes that exist in the human body are collectively known as the human microbiota, and this remarkably complex and poorly understood group of communities has an enormous impact on humans^[384]. The Human Microbiome Project, funded by the National Institutes of Health, aims to develop tools and databases for the research community to study the role of these microbes in human health and disease. One of the tasks the NIH has set itself is to develop a catalog of the microbial genome sequences of reference strains^[385]. For example, the microbiome diversity in the bronchial tracts of patients with chronic obstructive pulmonary disease has been documented^[386]. More advances in understanding the pathogenesis of *A. baumannii* using the databases of the Human Microbiome Project can be anticipated.

In conclusion, we hope that this review will aid in understanding the relevant studies regarding the antimicrobial resistance of *A. baumannii* as well as the currently available treatment options for the infections that this pathogen cause, thereby leading to new strategies to combat *A. baumannii*.

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Follicular contact dermatitis revisited: A review emphasizing neomycin-associated follicular contact dermatitis

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Abstract

Follicular contact dermatitis clinically presents as individual papules that include a central hair follicle. Pathologic features involve the follicle and the surrounding dermis: spongiosis and vesicle formation of the follicular epithelium associated with perifollicular and perivascular lymphocytic inflammation. Using the PubMed database, an extensive literature search was performed on follicular contact dermatitis and neomycin. Relevant papers were reviewed and the clinical and pathologic features, the associated chemicals (including a more detailed description of neomycin), the hypothesized pathogenesis, and the management of follicular contact dermatitis were described. Several agents—either as allergens or irritants—have been reported to elicit follicular contact dermatitis. Several hypotheses have been suggested for the selective involvement of the follicles in follicular contact dermatitis: patient allergenicity, characteristics of the agent, vehicle containing the agent, application of the agent, and external factors. The differential diagnosis of follicular contact dermatitis includes not only recurrent infundibulofolliculitis, but also drug eruption, mite infestation, viral infection, and dermatoses that affect hair follicles. The primary therapeutic intervention for follicular contact dermatitis is withdrawal of the causative agent; treatment with a topical corticosteroid preparation may also

promote resolution of the dermatitis. In conclusion, follicular contact dermatitis may be secondary to allergens or irritants; topical antibiotics, including neomycin, may cause this condition. Several factors may account for the selective involvement of the hair follicle in this condition. Treatment of the dermatitis requires withdrawal of the associated topical agent; in addition, topical corticosteroids may be helpful to promote resolution of lesions.

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Key words: Allergic; Contact; Dermatitis; Follicular; Irritant; Neomycin; Papular

Core tip: Follicular contact dermatitis can be elicited by several agents and clinically presents as individual papules that include a central hair follicle. Pathologic features involve the follicle and the surrounding dermis. Hypotheses for the selective involvement of the follicles include patient allergenicity, characteristics of the agent, vehicle containing the agent, application of the agent, and external factors. The differential diagnosis includes dermatoses that affect hair follicles, drug eruption, infundibulofolliculitis, mite infestation and viral infection. Treatment with a topical corticosteroid preparation and/or withdrawal of the causative agent are therapeutic interventions for follicular contact dermatitis.

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INTRODUCTION

Contact dermatitis can be either allergic or irritant in eti-

ology. Follicular contact dermatitis is a variant of contact dermatitis that has been observed in individuals secondary to incidental exposure or patch testing to the eliciting agent. The allergens and irritants that have previously been reported to cause follicular contact dermatitis are summarized and neomycin-associated follicular contact dermatitis is emphasized.

CLINICAL MORPHOLOGY AND SYMPTOMS OF FOLLICULAR CONTACT DERMATITIS

Follicular contact dermatitis is usually characterized by individual papules that include a central hair follicle. However, prominent hairs within the papules may not be readily visible when the lesions surround vellus hairs^[1]. The papular lesions are frequently pruritic and occasionally painful or burning. The individual lesions have also been described as poral^[2,3] or acneiform^[1]. In addition, the clinical spectrum of follicular contact dermatitis also includes follicular-based pustules^[3].

PATHOLOGY OF FOLLICULAR CONTACT DERMATITIS

Microscopic examination of the perifollicular papule is similar, regardless of the eliciting contactant. The pathologic changes involve the follicle and the surrounding dermis. There is often spongiosis and vesicle formation of the follicular epithelium or the eccrine sweat ducts or both. In the dermis, predominantly lymphocytic inflammation is noted around the periadnexal vessels, the follicle and/or the eccrine pore. Importantly, the epithelium adjacent to the follicle or pore is normal in appearance^[1-12].

ALLERGIC CONTACT DERMATITIS AND TOPICAL ANTIBIOTICS

Allergic contact dermatitis to topical antibiotics is a relatively common phenomenon. The North American Contact Dermatitis Group reported that among patients referred for patch testing, during 1985 to 2004, the prevalence of allergic contact dermatitis to neomycin ranged from 7.2 to 13.1 percent^[13].

Allergic contact dermatitis to topical antibiotics is most commonly observed in certain at-risk populations. These include patients with chronic eczematous dermatoses (such as atopy and stasis dermatitis), chronic otitis externa, chronic venous insufficiency, and post operative or post traumatic wounds. In addition, an occupational risk to develop allergic contact dermatitis to antibiotics occurs more frequently in those individuals who handle them regularly, such as farmers, health care workers, pharmaceutical employees, and veterinary surgeons^[13].

CHEMICALS CAPABLE OF ELICITING FOLLICULAR CONTACT DERMATITIS

Several chemicals, including topical antibiotics, have been described in either individual reports or larger studies to elicit follicular contact dermatitis. The agents associated with the development of follicular contact dermatitis can be allergens (Table 1)^[1-10,14-21] or irritants (Table 2)^[4,5,11,22-28]. Several metals have been associated with follicular patch test reactions: chromium, cobalt, copper, fluoride, and nickel^[3,29]. Allergic and non-allergic development of follicular contact dermatitis has also been observed following exposure to tocopheryl linoleate, a vitamin E derivative^[4,5].

Neomycin-associated follicular contact dermatitis

Neomycin-drug characteristics: Neomycin is produced by the growth of *Streptomyces fradiae*. It is an aminoglycoside antibiotic. Its efficacy as an antimicrobial is based upon the drug's ability to irreversibly bind to the 30S ribosomal RNA subunits and inhibit bacterial protein synthesis^[13,30-32].

Neomycin can be used as a topical antibiotic and has activity against many aerobic Gram-negative organisms (except *Pseudomonas aeruginosa*). It is also effective against some aerobic Gram-positive bacteria including *Staphylococci*. However it is not effective against *Streptococci*^[13,30-32].

Neomycin is usually formulated commercially as 20% neomycin sulfate in a petrolatum vehicle. However, it is often combined with other topical antibiotics such as bacitracin zinc and polymyxin B sulfate. This is done to expand the antimicrobial coverage^[13,30-32].

Neomycin-clinical presentation: The woman in Figures 1-4 developed follicular contact dermatitis to an antibiotic ointment that contained neomycin sulfate in combination with bacitracin zinc and polymyxin B sulfate. Indeed, individual hair follicles were observed in the center of the papular lesions (Figure 4). Allergic contact dermatitis has been reported to all three components of this antibiotic^[33-35]. However, follicular contact dermatitis has only been described in association with neomycin.

Neomycin-prior observations: Allergic contact dermatitis to neomycin was initially reported in 1952^[36]. Six years later, in 1958, Epstein^[9] described contact dermatitis to neomycin as "...an aggravation or "irritation" of a pre-existing dermatitis..." and not the obvious picture of an acute contact dermatitis. He considered it to represent a dermal contact sensitivity reaction^[9]. The lesions elicited by patch testing clinically presented as papules and histologically demonstrated an intact epidermis with pathologic changes in the dermis^[9].

Subsequently, Jillson *et al*^[7] reported contact dermatitis to neomycin in 10 patients with atopic dermatitis. One of the patients, a 50-year-old woman had an eczematous dermatitis of her left flexor arm for which prior treatment with neomycin ointment had irritated the dermati-

Table 1 Agents associated with allergic follicular contact dermatitis

Agent	Comment	Ref.
Ammonium fluoride	A farm helper who sprayed trees with chemical and had an exudative dermatitis and a postal employee with right foot and bilateral popliteal dermatitis; patch test showed folliculoporal reaction	[2]
Chromium trioxide	A shoe-shiner with severe hand dermatitis, a plasterer who worked with cement (after a cast had been applied to his hand to treat a fracture), and an electrician with chronic dermatitis flared when he drilled through aluminum coated with zinc chromate primer; all had a folliculoporal patch testing reaction	[2]
Cobalt chloride	103 follicular patch test reactions in 853 heavy metal workers that were tested	[3]
Colored permanent pressing sheets chemical	Sheets were 50% cotton and 50% polyester; widely disseminated erythematous follicular keratotic papules; primarily on hairy areas with a predominance on legs and forearms. Several washings of sheets did not prevent dermatitis; it persisted up to 8 wk after sheets removed	[10]
Copper sulfate	110 patients patch tested; 8 of 69 who reacted had follicular or poral (folliculoporal) reactions	[2]
Cosmetic creams	5 young women in a 3 mo period; at sites where cream applied following bathing or before sun exposure: extensor limbs (with well developed vellus hairs) were greatly affected	[1]
Dander (human)	Patch test reactions to dander histologically showed eczematous changes in the upper parts of hair follicles and clinically consisted of erythema and papules; they were positive in 120 of 181 atopic patients, 2 of 28 allergic contact dermatitis patients, and 1 of 31 normal controls	[14]
Formaldehyde	A postal employee with right foot and bilateral popliteal dermatitis; patch test showed folliculoporal reaction 2 women developed textile contact dermatitis to a new long sleeved shirt and new pajamas; a hair usually pierced the center of the papular lesions Positive patch test reactions frequently showed a follicular pattern; in some patients, only bright red follicular papules set in a background of normal appearing skin	[2,8,15]
Homomenthyl salicylate	Sunscreening chemical in a suntan lotion; 2 women with follicular dermatitis. One of the woman developed consort allergic contact dermatitis from contact with her boy friend who used the lotion; she was originally misdiagnosed as having recurrent disseminated infundibulofolliculitis	[6]
Methyl glucose sesquistearate	Follicular dermatitis developed to both a lotion and facial cream that contained this chemical	[16]
Neomycin	Repeat topical application on abdomen (current report) and patch test reaction (woman with atopy and left arm dermatitis that flared after applying neomycin ointment	[7,9]
Nickel sulfate	A farm helper who sprayed trees with chemical and had an exudative dermatitis; patch test showed folliculoporal reaction 29 follicular patch test reactions in 853 heavy metal workers that were tested Female production line worker with dermatitis of hands, chest and face after exposed to metals and cutting fluids and patch test positive to nickel; she developed follicular contact dermatitis in her pubic area 2 d after shaving with a metal razor blade	[2,17,18]
Paraphenylenediamine	An atopic woman with recurrent episodes of follicular-based pruritic papules on her face, chest and back beginning 3 wk after starting daily oral hydrochlorothiazide; she had a similar dermatitis after contact with "black hair dye" and positive patch test reaction to paraphenylenediamine (which cross reacts with her new oral antihypertensive)	[19]
Polyoxyethylene laurylether	An emulsifier (and an addition of lauryl alcohol and ethylene oxide) used in cosmetics. A woman developed pruritic follicular facial papules after starting to use new cosmetics; both a use test and a patch test for polyoxyethylene laurylether showed a follicular papular reaction	[20]
Potassium dichromate	61 follicular patch test reactions in 853 heavy metal workers that were tested	[3]
Selenium salts	In glass industry, 4 employees exposed to barium and sodium selenite suffered from dermatitis and/or conjunctivitis; 2 of the patients developed follicular allergic contact dermatitis with papulo-follicular lesions. Patch testing with sodium selenite confirmed the diagnosis	[21]
Sodium tungstate	3 follicular patch test reactions in 853 heavy metal workers that were tested; heavy metal contains about 90% tungsten carbide	[3,18]
Tocopheryl linoleate	Vitamin E derivative added to base formulation of a cosmetic line in Switzerland; 905 patients with papular and follicular dermatitis. Positive patch test reactions to cosmetics and vitamin E linoleate	[4,5]

tis^[7]. Patch testing to neomycin ointment "...was characterized by multiple small (papules of) eczematous areas rather than a confluent eczematous plaque^[7]".

The patient in Figures 1-4 developed allergic contact dermatitis to neomycin. Her initial lesions were perifollicular papules. Some of these subsequently developed into confluent plaques.

PATHOGENESIS OF FOLLICULAR CONTACT DERMATITIS

Several hypotheses have been suggested for the selective involvement of the follicles in follicular contact dermatitis in contrast to the diffuse clinical changes more fre-

quently observed in allergic or irritant contact dermatitis. These include direct penetration of the stratum corneum by the agent *via* the pilosebaceous apparatus, hapten conjugation of the agent to a substance only present in the infundibular region, or both^[6]. Other factors may also influence the development of follicular contact dermatitis.

Patient allergenicity

Previously individuals with atopy were considered less likely to be susceptible to allergic contact dermatitis. However, several subsequent studies have demonstrated that atopic patients not only develop contact dermatitis to metals^[37], but also more commonly develop follicular contact dermatitis^[38,39]. Hence, the patient's diathesis to allergens may influence whether they develop follicular

Table 2 Agents associated with irritant follicular contact dermatitis

Agent	Comment	Ref.
Beetle toxin	Pederin toxin released as a defensive mechanism from the rove (staphylinid) beetle in hot tropical and moderate climate regions typically limited to uncovered body areas	[22,23]
Bis-hydroxyethyl-tallow amine	Antistatic agent used to impregnate plastic tote boxes; outbreak of the hand or arms of 48.3% (14 of 29) of employees of the incoming inspections department of a microelectronic plant. The chemical provoked both follicular and nonfollicular irritant dermatitis; it was also a potential skin sensitizer	[24]
Coal-tar products	Hand dermatitis presenting with follicular papules and pustules at the site of exposure to coal-tar oils, creosote, pitch	[25]
Croton oil	Occupational source for irritant pustular and follicular irritant contact hand dermatitis	[25]
Debromoaplysiatoxin	Occurs after swimming in water contaminated by sea algae (<i>Lyngbya majuscula</i> Gomont); the algae cause a seaweed dermatitis in persons swimming off the coast of Oahu, Hawaii. Topical application of the toxin produces an irritant pustular folliculitis	[26]
Fluorine	Antirust solution containing 20% ammonium bifluoride diluted in water; acute irritant contact dermatitis in an atopic child. Rusted buckles of the right shoe cleaned with solution; 12 h later, the 19-mo-old boy developed an erythematous pustular dermatitis on the areas of the treated buckles	[27]
Greases	Occupational source for irritant pustular and follicular irritant contact hand dermatitis	[25]
Naphthalenes	Occupational source for irritant pustular and follicular irritant contact hand dermatitis	[25]
Petroleum	Hand dermatitis presenting with follicular papules and pustules at the site of exposure to petroleum derivatives: crude oil and fractions, cutting oils; lesions develop at the contact site to oil-soaked and tar-soaked clothes	[25]
Propylene glycol	It is used as a solvent, a plasticizer, a component of household products, a food additive and an ingredient in cosmetics and pharmaceutical preparations. 45138 patients patch tested; only 1044 (2.4%) patients with actual allergic contact dermatitis and 43 (0.10%) patients with non-allergic follicular reactions	[28]
Tri-phenyl-tin-fluoride	It is a bioactive organo-tin compound used as agricultural fungicides, general biocides, bactericides, herbicides, insecticides and antifoulant in boat paints (ship bottom coatings); it is moderately toxic to the skin. The patient's forearm accidentally contacted an empty drum that was still contaminated with the chemical; within 2 d he developed multiple follicular keratosis-like red papules evenly distributed over the affected area	[11]
Tocopheryl linoleate	Vitamin E derivative added to base formulation of a cosmetic line in Switzerland; 905 patients with papular and follicular dermatitis. In a few patients, the skin reaction appeared after a few applications on discontinuous days or more rarely after a single application suggesting an irritation reaction	[4,5]



Figure 1 Neomycin-associated follicular contact dermatitis presenting as follicular papules on the right abdomen, in and around the umbilicus, and the suprapubic region. The patient is a 59-year-old Asian woman who presented with itchy lesions at the sites of prior incisions on her lower abdomen. Her past medical history was significant for stage I, T2N0M0 adenocarcinoma of the sigmoid colon. Her tumor was successfully managed by a laparoscopic anterior resection of the sigmoid colon.

contact dermatitis^[17].

Characteristics of the agent

Heavier molecules are less easily capable of penetrating the epidermis as compared to lighter molecules. Hence, it can be hypothesized that the heavier molecules exhibit a preference for entering the dermis through the pilosebaceous units of hair follicles. For example, cobalt demonstrates an increased number and severity of contact dermatitis reactions at follicles^[3]. Neomycin, is a larger

molecule than cobalt; therefore, the size of neomycin may account for the observed follicular contact dermatitis to this agent (Figures 1-4).

The concentration of the agent can also influence a predilection for follicular contact dermatitis. Not only cobalt, but also tungstate shows an increase in follicular reactions at higher concentrations^[3,18].

Vehicle containing the agent

Lipophilic irritant agents absorb through the pilosebaceous apparatus^[40]. However, water-soluble substances penetrate more easily into and around hair follicles^[3]. Yet, in patch test reactions to metals, follicular contact dermatitis is more common when the testing vehicle is petrolatum as compared to water^[3].

Application of the agent

Not only in patch testing, but also in clinical use features regarding the application of the agent can potentially influence the occurrence and severity of follicular contact dermatitis^[41,42]. It is reasonable to hypothesize that repeated application and occlusion of the agent may allow for greater contact with larger areas of epithelium instead of only the follicles, resulting in a more confluent dermatitis. Therefore, follicular reactions are less likely to occur when the agent is applied more frequently or is occluded.

External factors

Follicular contact dermatitis to heavy metals was increased in individuals with hyperkeratosis of their hair follicles;

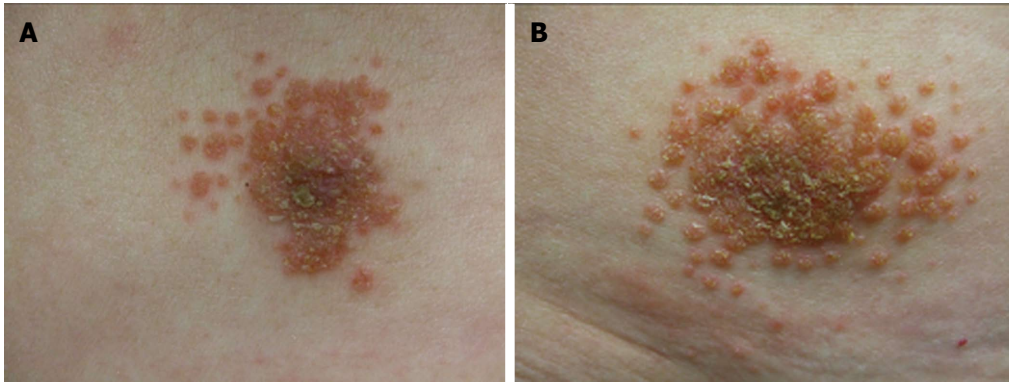


Figure 2 Closer view of neomycin-associated follicular contact dermatitis on the right mid abdomen (A) and right lower abdomen (B). The woman noted, one month postoperatively, that there was still some drainage from her surgical wounds. She was instructed to daily clean the sites and apply an antibiotic ointment that contained neomycin sulfate, polymyxin B zinc, and bacitracin zinc (Neosporin ointment). She began to develop small individual lesions at the sites of antibiotic ointment application after 6 wk of daily topical treatment; however, she continued to treat the incision sites for another 4 wk as the individual lesions enlarged and some become confluent-before seeking medical attention.



Figure 3 Cutaneous examination of her abdomen and suprapubic region (A) showed individual and confluent red-brown pruritic papules where she had been applying the antibiotic ointment to prior incision sites: right mid abdomen, right lower abdomen, umbilicus and periumbilical area (B, distant view and C, closer view) and suprapubic region.

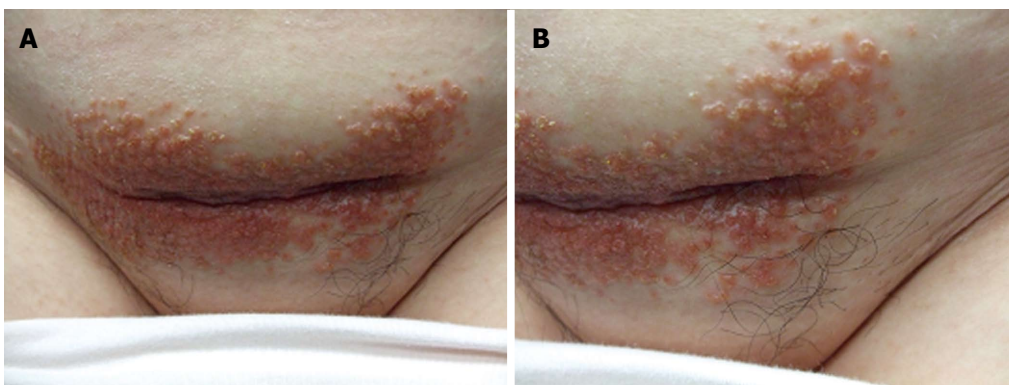


Figure 4 Distant (A) and closer (B) inspection, particularly of the lesion in her suprapubic area, showed individual hair follicles in the center of the papules. The topical antibiotic was discontinued and flucanide 0.05% cream was applied twice daily; all of the lesions resolved within 2 wk with residual post inflammatory hyperpigmentation at the sites.

however, it was not associated with either the presence of acne or sweating^[3]. In contrast, not only sweating, but also pressure and friction contributed to the development of follicular contact dermatitis caused by a chemical in colored permanent pressing sheets^[10]. These external factors enhanced the penetration of the allergen into the follicles of the patients who developed dermatitis^[10].

DIFFERENTIAL DIAGNOSIS OF FOLLICULAR CONTACT DERMATITIS

Conditions to be considered in the differential diagnosis of follicular contact dermatitis are listed in Table 3^[6,10]. Some of the patients with follicular contact dermatitis were initially considered to have disseminated recurrent

Table 3 Clinical differential diagnosis of follicular contact dermatitis

Drug eruption
Fiberglass dermatitis
Food allergy
Hyperkeratosis follicularis et follicularis in cutem penetrans (Kyle's disease)
Infundibulofolliculitis
Keratosis follicularis (Darier's disease)
Keratosis pilaris
Perforating folliculitis
Pityriasis rubra pilaris
Scabies
Viral exanthema

infundibular folliculitis-even though they were Caucasian^[6,10]. In contrast to follicular contact dermatitis which was characterized by severe itching or areas of erythema and oozing or both in some of the patients, infundibular folliculitis is typically observed in black patients as mild to moderately pruritic or burning, flesh colored, widely distributed, non-inflammatory follicular papules; the papules are typically refractory to treatment and the recurrent episodes persist for weeks to months before spontaneously resolving^[43,44].

An individual in whom infundibulofolliculitis was suspected presented with recurrent 2-mm erythematous follicular papules. She was a 24-year-old nurse whose skin eruption partially improved with topical corticosteroids and resolved when her boyfriend moved to another city. However, it recurred when he returned and they went to the beach. Subsequently, the diagnosis of consort follicular contact dermatitis to the homomenthyl salicylate in her boy friend's Coppertone sunscreen lotion was considered and confirmed by positive patch testing to the lotion; additional patch testing to each component of the lotion was only positive for homomenthyl salicylate^[6].

The other patients had been exposed to a chemical used in colored permanent-pressed sheets^[10]. Not only the distribution and duration of the follicular contact dermatitis, but also the histopathology of the chemical-associated lesions were similar to those observed in individuals with infundibulofolliculitis. However several features permitted the patients with follicular contact dermatitis to be differentiated from those with infundibulofolliculitis: severe itching (as compared to mild or moderate pruritus), the presence of erythematous and even oozing areas (as compared to noninflammatory lesions) and a white patient population (as compared to occurring in African American individuals)^[10].

MANAGEMENT OF FOLLICULAR CONTACT DERMATITIS

The primary management of follicular contact dermatitis is withdrawal of the causative agent. The skin lesions for many of the affected individuals either resolved spontaneously or following treatment with a topical corticosteroid preparation. However, in some of the patients

lesions either persisted or recurred even after elimination of the inducing chemical or repetitive washing of the eliciting item from the source of exposure; specifically, follicular contact dermatitis persisted up to 8 wk after exposure to chemical in colored permanent-pressed sheets had been eliminated and new lesions would appear even after the sheets had been washed 3 or 4 times^[10].

CONCLUSION

Follicular contact dermatitis clinically presents as individual papules that include a central hair follicle. Pathologic features involve the follicle and the surrounding dermis: spongiosis and vesicle formation of the follicular epithelium associated with perifollicular and perivascular lymphocytic inflammation. Several chemicals, including topical antibiotics, can elicit follicular contact dermatitis-either as allergens or irritants. Neomycin-associated follicular contact dermatitis was initially reported in 1952. Subsequently, follicular contact dermatitis in additional patients treated with neomycin was observed and the diagnosis was confirmed by patch testing with the agent. Several hypotheses have been suggested for the selective involvement of the follicles in follicular contact dermatitis: patient allergenicity, characteristics of the agent, vehicle containing the agent, application of the agent, and external factors. The differential diagnosis of follicular contact dermatitis includes not only recurrent infundibulofolliculitis, but also drug eruption, mite infestation, viral infection, and dermatoses that affect hair follicles. Withdrawal of the causative agent is the primary therapeutic intervention for follicular contact dermatitis. In addition, treatment with a topical corticosteroid preparation may promote resolution of the dermatitis.

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Prognostic factors in periodontal therapy and their association with treatment outcomes

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advancements on this topic have been made in the periodontal literature during the last decade. Current evidence shows that except for good prognosis, the assignment of overall prognosis remains rather dicey. The major focus of future studies should be to construct simplified prognostic models with high predictability that will increase the confidence of Dentists and Periodontists when assigning teeth prognosis.

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Abstract

During the incipient steps of periodontal treatment, clinicians are usually asked to predict the prognosis of teeth with compromised periodontium. The aim of this literature review was to investigate the association between periodontal Prognosis, Tooth Loss and risk indicators, such as smoking and genetics. Results showed that the definition of good prognosis has much higher predictability than the one for questionable prognosis. Several risk indicators for periodontal prognosis and tooth loss are discussed as well as different definitions of questionable prognosis and their success in predicting tooth loss. In conclusion, the major focus of future studies should be to construct simplified prognostic models with high predictability that will increase the confidence of dentists and periodontists when assigning teeth prognosis.

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Key words: Periodontal prognosis; Tooth loss; Risk indicators; Periodontitis

Core tip: During the incipient steps of periodontal treatment, clinicians are usually asked to predict the prognosis of teeth with compromised periodontium. Little

INTRODUCTION

During the incipient phases of periodontal treatment, clinicians are usually asked to predict the prognosis of teeth with compromised periodontium^[1]. To address this difficult and challenging task, Periodontists have introduced the term “questionable” prognosis. In essence, this term means that a tooth may or may not respond well to treatment and many factors such as patient/host susceptibility, age, location of the tooth and degree of bone loss among others must be weighted to better determine its prognosis.

Scientific attempts to identify risk indicators for tooth loss that can help clarify and better define this term have been reported in the literature. Usually retrospective and cross-sectional studies are employed as these types of investigations allow for access to a large pool of data for analysis without the cost, or the ethical limitations that pertain to interventional studies^[2]. The drawback is that it is uncertain whether an observational study can verify the causal role of a true risk factor, yet observational studies are valuable in identifying risk indicators^[2].

The aim of this critical review was to investigate the

association between periodontal Prognosis, Tooth Loss and risk indicators. This review paper will discuss how specific risk indicators affect periodontal prognosis and how accurate initial periodontal prognosis can be.

CRITICAL REVIEW

Inceptive definition of questionable prognosis

Hirschfeld *et al*^[3] (1978) presented data of a cross-sectional study that included 600 patients with at least 15 years and up to 50 years of follow-up. This patient cohort was described by the authors as consisting of well-motivated middle-class patients that attended frequent (4-6 mo intervals) recalls.

The authors allocated the patients in three groups based on their response to treatment: well maintained (83.2%), downhill (12.6%), or extreme downhill (4.2%).

In the well-maintained group a mean of 0.68 teeth per patient were lost during the follow-up. The number of teeth lost in the downhill and extreme downhill groups were 5.7 and 13.3 per patient, respectively.

In regards to risk indicators for post-treatment tooth loss, residual mobility was insignificant. The authors' definition of questionable prognosis (furcation involvement, deep non-eradicable pocket, extensive bone loss and/or at least grade 2 mobility with active inflammation) was accurate in depicting clinical reality for the well-maintained group. In this group 80% of the teeth lost had been initially assigned a questionable prognosis. That percentage dropped to approximately 50% in the remaining groups. Almost all of the teeth with questionable prognosis were lost in the extreme downhill group. The authors concluded that tooth loss patterns were case-related and they noted a bilaterally symmetrical pattern. They found a predictable order of likelihood of tooth loss according to position in the arch. Mandibular cuspids and first bicuspid responded most favorable to treatment and maintenance, while maxillary second and first molars and mandibular second and first molars were more susceptible to loss. In this study the characteristics of the extreme downhill group were not identified, so the question of how to predict which patients will lose more teeth remains unanswered.

In a similar study McLeod *et al*^[4] (1997) investigated the effectiveness of periodontal treatment in a cohort of patients with moderate to advanced periodontitis that were treated over a period of 29 years. Tooth loss was set as the primary outcome of treatment. The authors utilized the categorization to well-maintained (0-3 teeth lost), downhill (4-9 teeth lost) and extreme downhill (10-23 teeth lost) groups as suggested by Hirschfeld *et al*^[3] (1978). The authors defined moderate disease as 4-7 mm of CAL loss and severe as greater than 7 mm of loss. It should be expected that this patient pool would be assigned a diagnosis of severe periodontitis based on contemporary definitions. All patients were treated by means of SRP followed by surgical treatment if indicated and were put on frequent (3-6 mo) recalls. Again, the definition of questionable prognosis was based on Hirschfeld's definition^[3]. A total

of 2889 teeth were nested in the 114 patients included in this study. After a mean of 12.5 years post-treatment, 220 teeth were lost during maintenance. In agreement with the results of Hirschfeld *et al*^[3] (1978), the authors noted a bilateral pattern of tooth loss. They also noted that maxillary and mandibular molars and maxillary first premolars had a higher incidence of extractions. The distribution of patients in the well-maintained, downhill and extreme downhill groups was 84.2%, 13.2% and 2.6%, respectively. Those findings are in remarkable agreement with results from the study of Hirschfeld *et al*^[3] (1978). There was a higher rate of tooth loss in teeth with furcation involvement especially in the downhill groups. In regards to questionable prognosis accuracy, 529 teeth were initially assigned to this prognosis group. Sixty-eight of those teeth were lost (12.9%), while the remaining 152 teeth that were lost had not been assigned a questionable prognosis. Therefore, the negative predictive value of that definition of questionable prognosis is brought to question.

This study was novel in attempting to correlate the Hirschfeld *et al*^[3] (1978) classification of response to periodontal treatment with the AAP-accepted terminology for periodontal disease. According to the authors: "Nine of the 18 patients in the downhill and extreme-downhill groups had periodontal disease that would be classified as systemic-disease-associated or early-onset periodontitis, and the remaining patients would be classified as having refractory periodontitis". This statement is valuable as it provides an explanation for the response to treatment and maintenance, but does not yield information on how to predict which patients will have a downhill response.

McFall *et al*^[5] (1982), replicated the study of Hirschfeld *et al*^[3] (1978) in a faculty-practice based patient population ($n = 100$) that was followed up for at least 15 years. There results were in complete agreement with the previous studies. As in previous studies, maxillary 2nd molars were the most frequently lost teeth and mandibular cuspids and bicuspid were the less frequently lost ones. The definition of questionable prognosis that was utilized in this study predicted only 48.7% of the tooth loss in all groups. From the teeth that were initially assigned questionable prognosis, 62.3% were lost during the follow-up.

A question can be raised as to what benefit, other than a rough estimation of the percentage of poor responders, there is in categorizing patients in groups based on the outcome of treatment. Categorizing patients in a group prior to initiation of treatment, based on specific risk indicators and assessing the accuracy in predicting which patients will exhibit downhill response seems more reasonable.

Chace *et al*^[6] (1993) performed a cross-sectional study that specifically aimed to address the fate of teeth that were assigned a questionable prognosis. The authors' definition of questionable prognosis slightly differed from the previous definitions, as it required that the teeth simultaneously exhibited pocket depth greater than 6 mm, mobility greater than 0.5 mm in buccal-lingual direction, poor

root-crown ratio and at least class II furcation involvement. In this study 166 patients, lending 455 questionable teeth to the study, were followed up over up to 40 years. A total of 55 teeth were lost (12%), with an average survival period of 8.8 years. Half of the teeth were bilaterally symmetrical and as in the previous studies most frequently lost teeth mostly groups were maxillary second molars, first molars, first bicuspid, or mandibular molars^[3-5]. Even though the accuracy of the assignment of "questionable prognosis" could not be investigated with this study design, results of this study showed that teeth with significant loss of periodontal tissues could be functionally maintained. Yet, factors such as esthetics and patient satisfaction were not discussed.

In another study, Wilson *et al.*^[7] (1987) focused in investigating the effect of patient compliance on tooth loss. One hundred and sixty-two patients were followed up for at least 5 years and were categorized as "compliant", or "erratic". Results showed that completely compliant patients lost no teeth during the follow-up. Twenty-two patients in the erratic group lost a total 60 teeth, for an average of 0.06/patient/year in that group. The authors claimed a higher percentage of teeth with questionable to poor prognosis being lost, in comparison to teeth that were assigned good or fair prognosis, albeit no statistical test was performed.

AN EVIDENCED-BASED ATTEMPT TO DEFINE PROGNOSIS: THE "MCGUIRE AND NUNN" STUDIES

In 1991 McGuire^[1] evaluated the outcome of treatment in 100 patients that were followed up for a mean of 7 years following active treatment. All patients received standard of care non-surgical treatment and all of them received surgical treatment in areas with residual pockets. Patients underwent a stringent maintenance schedule with the first maintenance appointment scheduled at 1 mo post-surgery and at 1-3 mo intervals thereafter. Each tooth was assigned to one of the following five prognosis gradients: good, fair, poor, questionable, and hopeless. The author re-assigned prognosis to each tooth based on the clinical situation at 5 and 8 years post-active treatment. Results showed that the average prognosis of the teeth studied at each interval changed very little from initial to 5 to 8 years.

A 2.1% tooth loss (51/2484) was noted for the study population. The teeth with good prognosis remained relatively stable, while teeth in the fair and poor categories frequently improved. The questionable category generally got better, but a significant number of teeth were lost and teeth in the hopeless category were generally lost. Findings of interest were that prognosis was more accurate for single rooted teeth than multi-rooted, and that 3rd molars and mandibular molars tended to perform worse than expected. The author discussed that the criteria for assigning prognosis in this study were less lenient in downgrading a tooth to questionable prognosis in com-

parison to the criteria of Hirschfeld *et al.*^[3] (1978).

In the second part of this study the authors attempted to investigate the accuracy of a statistical model that would consider several explanatory variables such as, furcation involvement, pocket depth, percentage of bone loss, mobility, crown to root ratio and root proximity, based on the data published previously^[8]. The model was very accurate in predicting prognosis (approximately 80%), especially in non-molar teeth. When scrutinizing the results, the authors found that the accuracy of the model was significantly compromised when teeth with good prognosis were excluded from the analysis (< 50%). The clinical repercussion of those findings is debatable. It may not be as crucial to determine if a tooth that was assigned questionable prognosis may move to fair, or vice versa. On the contrary it is very valuable the ability to foresee which teeth will shift from the fair, or questionable gradient to hopeless. That question was addressed in the third part of this study that was published later the same year^[9].

In the third part of the study the authors extended the follow-up to 16 years. This extended observation time increased the number of teeth lost to 131 of the 2509 initially present. The average survival time for teeth that were lost was approximately 5 years post treatment. In this publication a true endpoint^[10] was chosen, tooth loss.

Results showed that both the sensitivity and specificity of the suggested prognosis classification increased when tooth loss was considered as the endpoint.

When questionable and hopeless prognoses were grouped together they were relatively accurate in predicting future tooth loss. The authors also constructed a proportional hazard model that identified initial probing depth, initial furcation involvement, initial mobility, initial percent bone loss, parafunctional habits with no biteguard, and smoking risk indicators for tooth loss.

CONTEMPORARY VIEWS ON PERIODONTAL PROGNOSIS

New data and studies have initiated a shift in the consideration of risk indicators for periodontal prognosis. A vastly increasing number of new studies are now focusing on risk indicators involving host susceptibility rather than local factors^[11,12]. The genetic and host components of periodontal disease and their association with periodontal prognosis are magnetizing the interest of clinicians. The pathophysiologic cascade underlining this relationship has not been clearly elucidated. Yet, there are clear indications of this association. Fardal *et al.*^[13] (2004) investigated risk factors associated with tooth loss due to periodontal reasons during maintenance phase of treatment in a hundred patients in a Norwegian specialist periodontal practice. This study examined how initial prognosis related to actual outcome as measured by a true point, namely periodontal tooth loss. The patients included in this practice-based study, had comprehensive periodontal treatment and were followed for 9-11 years

during maintenance care. The authors identified that only 36 (1.5%) of the 2436 teeth present at baseline were subsequently lost due to periodontal disease. The majority 27 (75%) of the teeth lost due to periodontal disease had been assigned an uncertain, poor or hopeless initial prognosis. Fardal *et al*^[13] found that tooth loss was significantly associated with older age (> 60 years), male gender and smoking, but was not significantly associated with oral health status and family history, and that compliance with maintenance following active periodontal treatment was associated with low levels of tooth loss. Notably, even though the majority of teeth lost due periodontal disease had been initially assigned an uncertain, poor or hopeless prognosis, 9 of the teeth lost (25%) had been assigned a good prognosis at baseline. This indicates that it is not always possible to identify all teeth that are at risk of being lost during the progression of periodontitis. From the interpretation of results of Fardal *et al*^[13] (2004) it is evident that risk indicators related with host and genetic components are more predictive of tooth loss, rather than those associated with clinical parameters and local factors. Age and gender were significantly associated with tooth loss in contrast with oral health status, indicating a strong association between tooth loss and the genetic-host component of periodontal disease^[13].

A common finding in earlier studies on tooth survival following active treatment and maintenance has shown that furcation involvement is a risk indicator for future tooth loss^[1,3,5] and makes assignment of accurate prognosis very challenging^[9]. Svärdröm *et al*^[14] (2000) evaluated 1313 molars in 222 patients in order to analyze the outcome of non-regenerative treatment. They found that from the 899 molars that were deemed maintainable, only 21 (3.5%) were extracted within a 10-year follow-up period. All molars in this group were treated with scaling and root planning followed by modified Widman flap surgery, if indicated. The authors concluded that molar teeth treated with non-resective, non-regenerative approaches have a good long-term prognosis if a frequent recall schedule is followed.

The potential prognostic value of clinical, genetic, and radiographic variables in predicting tooth loss in periodontal patients was assessed in a 10-year retrospective analysis^[15]. Sixty periodontal patients were treated according to the standard of care and were placed at 3-4 mo maintenance schedules. In addition to standard clinical and radiographic examination, the patient underwent interleukin-1 genotype assessment. The distance of the bottom of the bony defect to the root apex as well as molar teeth were significant predictors of tooth loss. On the contrary deep intrabony defects had a protective effect. Interleukin-1 test was not efficient as a predictor of tooth prognosis.

Faggion *et al*^[16] (2007) also attempted to identify risk indicators to construct a prognostic model. In agreement with the previous studies, teeth with multiple roots were identified as a significant factor. The authors also identified diabetes mellitus, reduced bone levels at baseline, non-vital pulp and tooth mobility as risk indicators for

future tooth loss.

A simplification of the McGuire^[1] (1991) classification of periodontal prognosis was proposed by Checchi *et al*^[17] (2002). This simplified classification includes three prognosis gradients: good, questionable, and hopeless. The authors elected to define prognosis based on residual bone levels and/or furcation involvement. Teeth with more than 75% per cent bone loss were assigned “hopeless” prognosis and teeth that had between 50% to 75% bone loss, or furcation involvement were assigned “questionable” prognosis. If a tooth exhibited both characteristics it was downgraded. Results showed that 0.07% of teeth with good prognosis were lost, 3.63% were lost from the questionable prognosis category and 11.34% were lost from the hopeless prognosis subgroup. While previous prognosis classifications were shown to be accurate for the “good” and “hopeless” prognosis, this simplified approach performed very well for “good” and “questionable” prognosis, but seemed to have been pessimistic in assigning “hopeless” prognosis.

Most of the studies mentioned in this review evaluated the prognosis of teeth that had undergone periodontal treatment and went into a maintenance phase. Neely *et al*^[18] (2001) looked into risk indicators for tooth loss in an untreated cohort of 154 Sri Lankan tea laborers. This patient cohort had no access to periodontal treatment and represented a population sample of untreated periodontal disease. Results were very interesting as they showed that plaque index and smoking were not associated with mean attachment loss. Age, gingival index, calculus index and time were associated with attachment loss over 20 years of follow-up.

The same group of researchers published a follow-up paper that evaluated a true endpoint (tooth loss) instead of a surrogate endpoint (attachment loss)^[19].

Results were striking as they significantly differed from results of the previous study. In this second part, none of the individual risk indicators had a significant impact on tooth loss. Tooth loss was associated with increasing attachment loss in the presence of use of betel nut. Interestingly, betel nut (a nut containing substances with vasoconstricting properties) was found to be a poor predictor of increase in attachment loss in the first part of the study. These findings point out that even though attachment loss is a well-established surrogate for tooth loss in the treatment of periodontitis, studies that utilize surrogate endpoints should always be reviewed with that limitation in mind^[20].

Other risk indicators for tooth loss

Several studies have investigated the effect of single risk indicators, or risk factors, depending on the definition, on tooth loss. Such ones are furcation involvement^[21], retained “hopeless” teeth^[22], residual deep pockets^[23] and maintenance schedule frequency^[24].

Axelsson *et al*^[24] (1981) assessed the efficacy of a stringent maintenance schedule in patients that had undergone surgical periodontal therapy. All patients were treated with modified widman flap surgery in all four

quadrants. One third of the initial group of 90 patients was referred back to their general dentists for maintenance, while the remaining two thirds underwent a stringent maintenance schedule that included professional debridement once every two months for the first two years post-operatively and once every three months thereafter. Results showed that there was a significant difference in pocket depth maintenance and maintenance of attachment levels around treated teeth between the “recall” and “non-recall” groups. It should be noted that patients in the stringent maintenance group received subgingival scaling at their bimonthly, or trimonthly visits, when indicated. Results on tooth maintenance in each group were not at all that impressive. No significant difference was noted in the number of teeth lost between the two groups. No teeth were lost in the “recall” group and only few teeth were lost in the “non-recall” group. Numerical results were not published. The authors concluded that stringent maintenance is of paramount importance as it can prevent future attachment loss. One could argue that the authors overemphasized results of this study. It is more reasonable to evaluate a true endpoint, such as tooth loss as being more significant over a surrogate, such as attachment loss^[10]. On the other hand the sample size on the non-recall group was smaller and as a result the study might have not been powered enough to identify a difference in the incidence of tooth loss, as this is a rare event.

Furcation involvement is another risk indicator that has been highlighted in several studies. Waerhaug^[21] (1980) investigated the anatomy and pathophysiology of furcation defects and concluded that if clinicians are aware of specific considerations when treating molar teeth with furcation defects, then their prognosis may be improved.

Interesting findings were that there is significantly increased attachment loss in the furcation area in comparison to the outer surfaces of the root and that the absence of bleeding on probing of the marginal gingiva is not associated with absence of inflammation, or progression of disease in the furcation area.

In a different study, researchers attempted to evaluate the retention of “hopeless” teeth as an indicator for future progression of disease in neighboring sites^[22]. In order to define “hopeless” the authors employed a combination of risk indicators such as at least 75% of bone loss, class 3 furcation defect, residual 8mm pocket depth, or repeated periodontal abscesses. Results showed no significant effect of the hopeless teeth on the “adjacent” surfaces of the neighboring teeth in comparison to the “non-adjacent” ones. Pocket depth post-treatment averaged at approximately 3.5 mm around the teeth that were in the vicinity of retained “hopeless” teeth, which indicates that even though no significant increase in surrogate markers for progression of disease was noted, many of the teeth had residual pockets greater than 3 mm. The authors concluded that retained “hopeless” teeth do not affect the periodontium of neighboring teeth as long as patients undergo frequent maintenance.

The influence of residual pockets in the prognosis of

teeth has also been a matter of interest. Matulienė *et al.*^[23] (2008) followed 172 patients with residual pockets after the active phase of treatment for 3-27 years. Progression of disease was defined as at least 3 mm of proximal attachment loss in at least two teeth. During the maintenance phase of treatment the percentage of pocket depths that were less than 5 mm did not change significantly. On the other hand, the percentage of pockets that had an initial depth of at least 5 mm increased from 2.9% to 4.3%. Increased pocket depth was found to be strongly associated with tooth loss in multilevel logistic regression analysis. During the follow-up 1.7 teeth were lost per patient. Residual pockets of at least 6 mm that were left untreated were a significant factor for tooth loss. During the maintenance phase, 43% of all cases were identified as progressing cases based on the definition mentioned above. The authors concluded that residual pockets with depth greater or equal to 6 mm represent incomplete periodontal treatment and are a risk indicator for tooth loss.

CONCLUSION

Even though this topic has been extensively discussed in the literature a solid definition of questionable prognosis has not been yet established. The importance of assigning an accurate prognosis for teeth prior to initiation of treatment cannot be emphasized enough. Not only it sets the foundation of trust between the therapist and the patient but also prevents legal implications from arising after the treatment process.

The major focus of future studies should be to construct simplified prognostic models with high predictability that will increase the confidence of dentists and periodontists when assigning teeth prognosis.

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Sleep disordered breathing in interstitial lung disease: A review

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Core tip: This article reviews the literature on sleep disordered breathing in interstitial lung disease, seeking to define the important contributing factors and sequelae. The key concepts that are explored include the contribution of nocturnal hypoxaemia to the development of pulmonary hypertension, and the mechanisms behind the observed high prevalence of obstructive sleep apnoea in interstitial lung disease patients.

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Abstract

Patients with interstitial lung disease commonly exhibit abnormal sleep architecture and increased sleep fragmentation on polysomnography. Fatigue is a frequent complaint, and it is likely that poor sleep quality is a significant contributor. A number of studies have shown that sleep disordered breathing is prevalent in this population, particularly in the idiopathic pulmonary fibrosis subgroup. The factors that predispose these patients to obstructive sleep apnoea are not well understood, however it is believed that reduced caudal traction on the upper airway can enhance collapsibility. Ventilatory control system instability may also be an important factor, particularly in those with increased chemo-responsiveness, and in hypoxic conditions. Transient, repetitive nocturnal oxygen desaturation is frequently observed in interstitial lung disease, both with and without associated obstructive apnoeas. There is increasing evidence that sleep-desaturation is associated with increased mortality, and may be important in the pathogenesis of pulmonary hypertension in this population.

INTRODUCTION

The interstitial lung diseases (ILD) are a heterogeneous group of disorders characterized by varying degrees of fibrosis and inflammation of lung parenchyma. Sufferers exhibit lung restriction and exercise intolerance, often developing progressive hypoxia over time. Independent of the presence of daytime hypoxia, many individuals with ILD are observed to desaturate during sleep, with or without associated apnoea.

There is mounting evidence that nocturnal hypoxia and sleep-disordered breathing (SDB) may contribute to adverse outcomes. Aside from resulting in poor sleep quality and daytime fatigue, transient repetitive desaturation and associated sympathetic nervous system activation may play a role in the development of pulmonary hypertension and contribute to increased mortality^[1-3].

Existing evidence on aspects of sleep physiology and pathophysiology in ILD will be considered within this review.

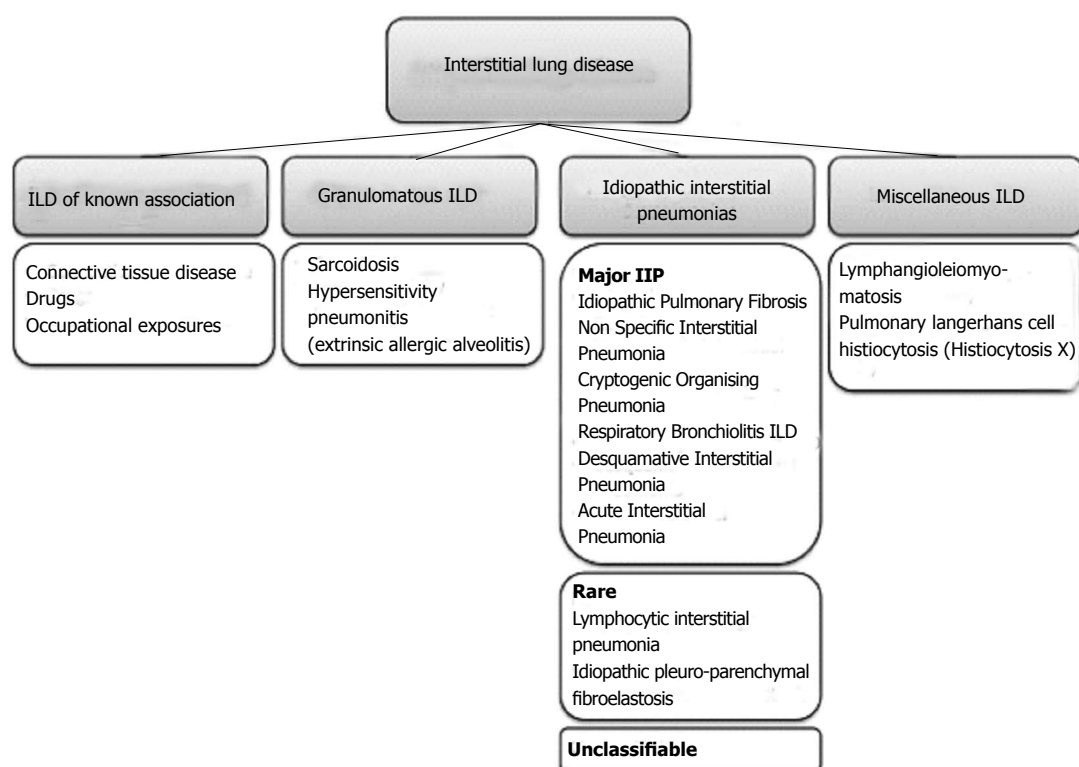


Figure 1 Classification scheme of interstitial lung disease (Adapted from^[4,5]). ILD: Interstitial lung disease; IIP: Idiopathic interstitial pneumonia.

POPULATION AT RISK

There are estimates of more than two hundred known causes of ILD, leading to restrictive physiology, dyspnoea and often a pervasive cough. These diseases can be divided into broad subcategories: (1) those with known aetiology such as the pneumoconioses, drug-related ILD, and connective tissue disease-associated ILD; (2) the granulomatous diseases such as sarcoidosis and chronic hypersensitivity pneumonitis; (3) the idiopathic interstitial pneumonias such as idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia, cryptogenic organizing pneumonia (COP); and (4) a miscellaneous group including pulmonary Langerhans cell histiocytosis^[4,5]. A classification scheme is depicted in Figure 1.

Many of these diseases evolve from an initial inflammatory process involving the lung interstitium with varying inclusion of the lung vasculature and airways. Over time, inflammation may give way to advancing fibrosis, especially in cases where diagnosis and treatment are delayed and inflammation persists unabated. In contrast, IPF is a distinct entity, in which inflammation does not appear to play an important role in the pathogenesis of fibrosis, which often progresses rapidly and relentlessly.

Irrespective of underlying aetiology, advancing fibrosis often leads to worsening gas exchange across a thickened collagen-dense interstitium, and respiratory failure may eventually ensue. Hypoxic vasoconstriction, endothelial remodelling and vascular obliteration all contribute to the development of pulmonary hypertension, a poor prognostic feature when present in ILD patients^[6,7].

SLEEP CHARACTERISTICS IN ILD

Breathing pattern during sleep

During wakefulness, ILD patients are known to have a rapid, shallow breathing pattern both at rest and with exercise^[8,9]. This is thought to be due to increased intrinsic elastic loading of respiratory muscles and stimulation of peripheral mechanoreceptors^[8,10]. Even in the face of severely impaired gas exchange, this respiratory pattern allows ILD patients to maintain ventilation and daytime eucapnia until very advanced stages of disease. During sleep, some investigators have found respiratory frequency, f to be no different to that when awake^[11-14]. Others have shown that f falls, but with an attendant increase in tidal volume, so that overall, the increased minute ventilation is preserved during sleep^[15].

Sleep architecture and sleep quality

Not surprisingly, sleep quality is comparatively poorer than that of the normal population. Nocturnal cough, medications, breathing difficulties, hypoxia and obstructive apneas have all been implicated in disrupting sleep in this population. Perez-Padilla *et al*^[14] in 1985, compared 11 ILD patients with age- and sex-matched controls. They reported decreased amounts of rapid eye movement (REM) sleep as well as significant sleep fragmentation in the patient group^[14]. Further prospective studies, with particular focus on IPF confirm these findings, and also report increased stage 1 and 2 sleep, reduced slow wave sleep and poorer overall sleep efficiency^[13,15-20]. Sleep characteristics in ILD subjects are shown in Table 1.

Table 1 Sleep characteristics in interstitial lung disease

Sleep characteristic	Abnormality	Patient group	Ref.
Respiratory rate	Decreased	ILD	[15]
	Unchanged	ILD, IPF	[11-14]
Stage 1/2 sleep	Increased	ILD	[14]
		IPF	[19,20]
REM sleep	Reduced	ILD	[11,14,15,25]
		Scleroderma	[26]
		IPF	[2,19,20]
Slow wave sleep	Reduced	IPF	[13]
		ILD	[17,25]
Arousal index	Increased	ILD	[12,14,15]
		IPF	[2,19,20]
Sleep efficiency	Reduced	ILD	[25]
		IPF	[13,19,20]

ILD: Interstitial lung disease; IPF: Idiopathic pulmonary fibrosis; REM: Rapid eye movement.

Daytime symptoms and quality of life

The symptoms of sleepiness and fatigue often co-exist in ILD patients. Common causative factors include systemic inflammation, treatment side effects, age and comorbidities. Depression and disease-related stressors also affect many. In addition, sleep fragmentation appears to be a substantial contributor, as demonstrated in studies using numerous sleep and health-related quality of life (QoL) questionnaires in ILD subjects. Fatigue is frequently reported, impacting on wellbeing and daytime function^[13,21,22]. The Epworth Sleepiness Scale (ESS), and Pittsburgh Sleep Quality Index (PSQI) scores are higher in unselected ILD patients than in normal controls, indicating poorer quality sleep^[22-24]. However, in ILD cohorts with polysomnography, the ESS and other tools do not reliably predict severity of sleep-disordered breathing^[2,19,25]. The PSQI does appear to correlate with health-related QoL indices, particularly physical function and vitality, highlighting the pervasive influence of sleep fragmentation^[22]. Nocturnal hypoxia in ILD patients is also independently associated with a reduction in energy levels, as well as physical and social functioning, as assessed by a variety of QoL instruments^[13,21].

Non-respiratory disturbances to sleep

Increased periodic limb movements and restless legs syndrome (RLS) have been documented in IPF and scleroderma patients^[17,26]. A self-reported study in IPF patients and normal controls, however, did not find any difference in the incidence of RLS^[27]. Gastro-oesophageal reflux disease may also play a role in sleep disruption, particularly in high-risk groups including scleroderma patients^[26].

OBSTRUCTIVE SLEEP APNOEA IN ILD

Increasing attention has been focused on the prevalence of obstructive sleep apnoea (OSA) in ILD, with much of the cross-sectional data coming from studies in IPF patients. Three prospective studies showed the incidence of OSA in IPF subjects to markedly exceed that reported in healthy age-matched populations, with estimates between

59% and 90%^[2,19,20]. This increased risk however, does not appear to be unique to IPF, with studies of mixed ILD populations (and in particular sarcoidosis and scleroderma subgroups) demonstrating similar findings^[18,25,28]. These results are summarized in Table 2.

Association between degree of OSA and severity of lung disease

Although retrospective analyses of ILD subjects suggest an association between the degree of lung restriction and the risk and severity of sleep disordered breathing, this has not been demonstrated in larger, prospective studies^[2,17,19,20,25,29]. The only correlation between measured lung volumes and PSG parameters, reported by Mermigkis *et al.*^[20], was in total lung capacity and REM sleep apnoea hypopnoea index (AHI). Kolilekas *et al.*^[2] found an association between overall AHI and peak VO₂ on exercise testing, but this may simply reflect poorer daytime function and more deconditioning in those with more fragmented sleep.

Hypotheses for why SDB is increased in patients with ILD

In the general OSA literature, recent attention has been turned towards the inherent characteristics that predispose individuals to sleep disordered breathing. The two key components believed to underscore the pathogenesis of OSA are increased upper airway collapsibility and enhanced ventilatory control system instability^[30]. It is useful to consider these processes in the ILD population.

Upper airway collapsibility: It is generally believed that restrictive lung disease leads to increased upper airway collapsibility through reduced caudal traction on these structures^[31,32]. The inability to demonstrate a relationship between lung function parameters and AHI seems to refute this theory. One limitation with all reported data, however, is the assessment of lung function in the upright position only. There may be more robust associations between supine measurements and SDB, but this is yet to be investigated in ILD subjects. Increased body mass index (BMI) is associated with deposition of adipose tissue around upper airway structures, enhancing collapsibility. BMI correlates with AHI in some, but not all studies of ILD subjects, suggesting that other mechanisms are important^[2,18-20,25].

Ventilatory control system instability: In sleep, any rise in arterial CO₂ (such as occurs with an apnoea) will stimulate carotid and medullary chemoreceptors, resulting in a central respiratory motor output response^[33]. The direct activation of respiratory pump muscles and upper airway dilator muscles (*e.g.*, genioglossus) restores upper airway patency, and is often accompanied by an arousal. Some individuals with heightened chemo-responsiveness may overshoot the eucapnic range, by overventilating in response to apnoea-induced hypercapnia. The ensuing hypocapnia will then cause a further apnoea, sometimes leading to a cyclical pattern of repetitive apnoeas as the ventilatory system continues to attempt to achieve ho-

Table 2 Prevalence of obstructive sleep apnoea in interstitial lung disease populations *n* (%)

Ref.	Population	Prevalence of OSA	M/F	Age (mean)	BMI (mean)	Comment
Aydoğdu <i>et al</i> ^[118] , 2006	ILD	24 (65)				Abstract only
Mermigkis <i>et al</i> ^[117] , 2007	IPF	11 (61)	12/6	68.1	33.2	Retrospective study; subjects with symptoms of SDB
Lancaster <i>et al</i> ^[119] , 2009	IPF	44 (88)	34/16	65.7	32.2	Prospective study of unselected patients; 16 subjects used oxygen during PSG
Mermigkis <i>et al</i> ^[120] , 2010	IPF	20 (59)	21/13	65.0	27.3	Prospective study of subjects with newly diagnosed IPF
Kolilekas <i>et al</i> ^[121] , 2013	IPF	28 (90)	24/7	68.0	28.7	Increased AHI associated with decreased survival, after exclusion of those prescribed CPAP
Pihtili <i>et al</i> ^[125] , 2013	ILD	34 (68)	14/36	53.9	25.9	Prospective study; excluded obese subjects (BMI ≥ 30)
	IPF	14 (82)				
	Sarcoidosis	10 (67)				
	Scleroderma	10 (56)				

OSA: Obstructive sleep apnoea; BMI: Body mass index; ILD: Interstitial lung disease; IPF: Idiopathic pulmonary fibrosis; SDB: Sleep disordered breathing; PSG: Polysomnography; AHI: Apnoea hypopnoea index; CPAP: Continuous positive airway pressure.

meostasis. This ventilatory system instability is believed to be an important contributing factor in many with OSA^[34].

Intermittent hypoxia enhances chemo-responsiveness, and is likely to exacerbate ventilatory instability in susceptible individuals^[35]. This may at least partly explain the frequent observation in some ILD patients, where repetitive apnoeas are unmasked during REM sleep when hypoxia is most pronounced^[20].

NOCTURNAL OXYGEN DESATURATION IN ILD

Nocturnal hypoxia is common in ILD, both with and without concomitant OSA. The relative importance of this has been debated, with some early studies concluding that desaturation was minimal, having little clinical impact in ILD^[15,16,36]. Other observational studies found that ILD patients experience transient or sustained hypoxia repetitively throughout sleep, leading to a substantial cumulative period of time with SpO₂ below 90%^[11-14,18,21]. More recently, sleep-desaturation has been found to be an independent predictor of poorer prognosis^[1,2].

Whilst obstructive events will undoubtedly be the cause for a proportion of the transient desaturations, there are many other pathophysiologic contributions. Perez-Padilla *et al*^[14], did not observe OSA in any of their subjects, but transient desaturation was a frequent occurrence amounting to an average of nearly 50% of total sleep time with SpO₂ below 90%. Further studies found the degree of desaturation that subjects experienced during sleep was independent from oxygen desaturation during moderate and maximal exercise^[2,11,16].

There are a number of reasons why ILD patients might be more vulnerable to desaturation during sleep than normal subjects, and indeed than sufferers of OSA with normal lungs. Firstly, many patients will be on the steep portion of the oxygen-haemoglobin dissociation curve, whereby small changes in arterial oxygen tension lead to large decrements in saturation. In support of this is the observation of greater degrees of sleep desaturation in those with lower awake resting PaO₂ and

SaO₂^[16,21,36,37]. Hypoxia may also occur due to worsening ventilation/perfusion inhomogeneity, and also alveolar hypoventilation, particularly during REM sleep^[3]. Findley *et al*^[38] studied the effect of lung volume on apnoea-related desaturation in normal subjects lying supine. Apnoeas were initiated at a range of lung volumes between total lung capacity and residual volume. The most severe desaturations occurred with apnoeas at the lower volumes, presumably because of the greater relative impact of dependent airway closure. This is a further mechanism of sleep-related hypoxia that may be extrapolated to individuals with lung restriction from ILD and concomitant sleep apnoea.

Predicting nocturnal desaturation in ILD patients

As might be expected, daytime hypoxia has been identified as a predictor of night-time desaturation in a number of studies^[16,21,36,37]. Severity of lung restriction and degree of desaturation with exercise, on the other hand, do not correlate well with nocturnal hypoxia^[1,16,21,37,39]. Respiratory drive during wakefulness, (measured as the change in ventilation in response to changes in PaCO₂), shows a tight negative correlation with the degree of desaturation in both REM and NREM sleep in ILD patients^[37], suggesting the innate chemo-responsiveness of the individual will also influence susceptibility to hypoxia. It is possible also that repeated severe episodes of nocturnal desaturation will eventually blunt this responsiveness, further perpetuating the problem as the disease advances.

Oxygen supplementation during sleep

Although there is widespread practice to provide supplemental oxygen for chronic lung disease patients with significant sleep desaturation, there is very little supportive evidence. In the COPD literature, the survival benefit derived from continuous oxygen therapy has not been demonstrated with overnight supplementation in those with nocturnal hypoxia only^[40-42]. There is some suggestion that pulmonary haemodynamics may be improved in COPD patients with long-term nocturnal oxygen^[43].

Only two studies have looked at the acute use of noc-

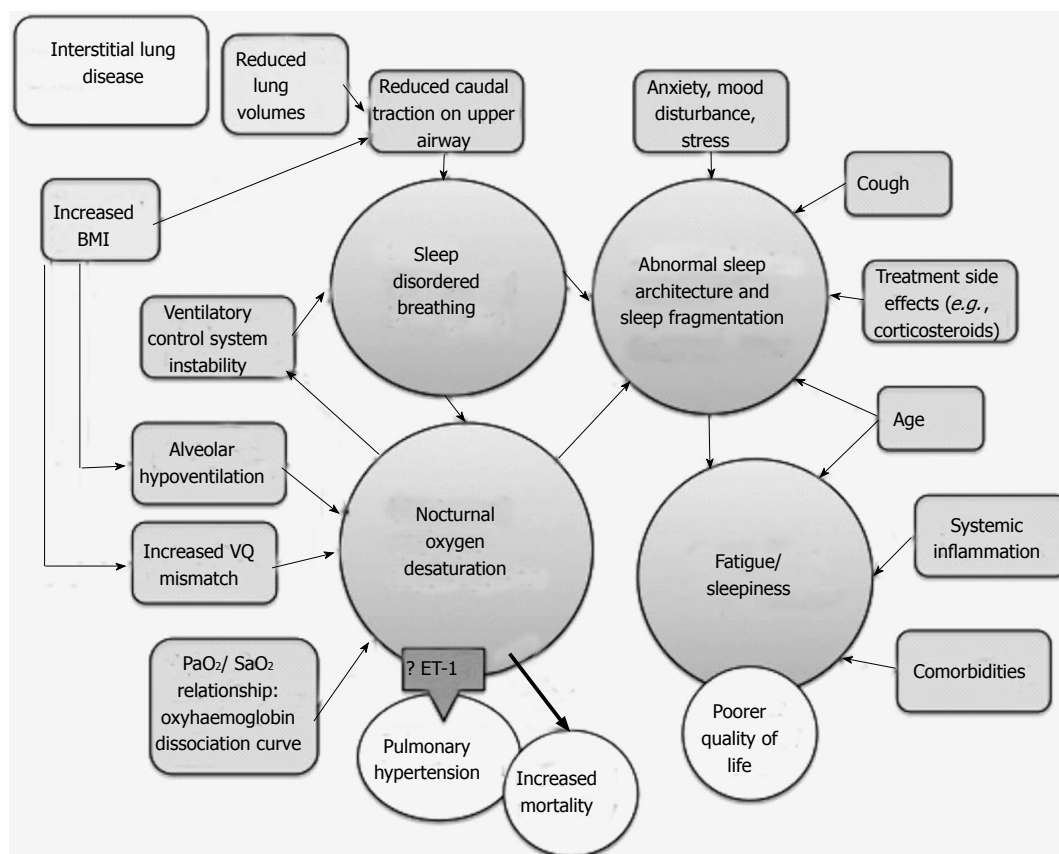


Figure 2 Mechanisms of sleep-related pathology in interstitial lung disease. BMI: Body mass index; VQ: Ventilation-perfusion; ET-1: Endothelin-1.

turnal oxygen in ILD. By eliminating sleep-hypoxia with supplemental oxygen, Shea and co-workers were able to demonstrate a fall in both f and minute ventilation compared with awake values, approximating those of normal controls^[44]. Vazquez studied ILD subjects at 2240 m above sea-level, breathing air or oxygen on two separate nights^[45]. Not surprisingly, all were hypoxic at rest (mean PaO_2 51 mmHg). With the addition of low-flow oxygen during sleep, heart rate and f were reduced and oxygenation improved. Sleep architecture, efficiency and arousal index were not significantly altered. To date, there have been no studies to address whether sleep quality or haemodynamics may improve with long-term use in ILD.

NOCTURNAL DESATURATION AND PULMONARY HYPERTENSION

The link between intermittent desaturation and increased pulmonary arterial pressure was hypothesised nearly forty years ago^[46,47]. Cross-sectional data in ILD subjects confirms the association between severity of nocturnal desaturation and the presence of pulmonary hypertension on echocardiography and right heart catheterisation^[1,39,48]. Furthermore, prolonged exposure to transient, repetitive hypoxia in both animals and humans leads to measurable changes in pulmonary haemodynamics^[49]. Tatsumi and colleagues studied subjects with both obstructive and restrictive lung diseases, comparing those with significant

nocturnal oxygen desaturation (NOD) to those without NOD, but matched for other disease variables^[3]. Daytime, supine pulmonary arterial pressures (PAP) and pulmonary vascular resistances (PVR) were significantly higher in the NOD group. Under hypoxic conditions, these differences became more marked. Hyperoxia, on the other hand, improved PVR and PAP, but not to the normal range seen in the non-NOD patients. This experimental data suggests that the effects may last well beyond the acute period, due to permanent structural changes in the vasculature.

Biomarkers in pulmonary hypertension

Serum Endothelin-1 (ET-1), a vasoactive peptide believed to be important in the pathogenesis of pulmonary hypertension, has been measured in ILD patients during sleep, in a novel study by Trakada and colleagues^[50]. During wakefulness, ET-1 was significantly higher in those with elevated pulmonary pressures. During sleep, ET-1 rose acutely in all patients during episodes of desaturation below 90%, and correlated with simultaneously measured PaO_2 concentrations and pulmonary arterial pressures. This very interesting research highlights a putative mechanistic pathway in the evolution of pulmonary vascular disease in ILD patients.

In summary, there is increasing evidence that nocturnal desaturation is not a benign phenomenon in ILD patients. In a large proportion, NOD occurs transiently and repeatedly throughout sleep, both with and without associated apnoeas. This may promote development of

pulmonary hypertension, and is independently associated with higher mortality. Mechanisms of SDB and NOD are illustrated in Figure 2.

CONCLUSION

A small but growing body of literature suggests that SDB and NOD are common in patients with IPF and other ILD. There is still much to learn regarding the true impact that these have on the natural history of disease. A large area of uncertainty remains in whether targeted treatment (*e.g.*, positive pressure ventilation, oxygen or other novel therapies) will offer any quality of life or mortality benefits.

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Role of MGMT as biomarker in colorectal cancer

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includes investigation of synergic combinations with other agents such as fluoropyrimidines and research for additional biomarkers, in order to better define the role of temozolomide in the treatment of individual patients.

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Key words: Colorectal cancer; O⁶-methylguanine DNA methyltransferase; Temozolomide; Dacarbazine; Biomarker

Core tip: O⁶-methylguanine DNA methyltransferase (MGMT) methylation is involved in colorectal carcinogenesis and represents a predictive biomarker for alkylating agents in metastatic colorectal cancer. In fact, patients with chemorefractory metastatic colorectal cancer with MGMT methylation derived promising response from treatment with dacarbazine or temozolomide, and ongoing research is investigating the efficacy of temozolomide in combination with other chemotherapy drugs for MGMT-methylated colorectal cancer. Future challenges include the combination with biologic drugs and the research for additional biomarkers.

Abstract

O⁶-methylguanine DNA methyltransferase (MGMT) gene promoter methylation plays an important role in colorectal carcinogenesis, occurring in about 30%-40% of metastatic colorectal cancer. Its prognostic role has not been defined yet, but loss of expression of MGMT, which is secondary to gene promoter methylation, results in an interesting high response to alkylating agents such as dacarbazine and temozolomide. In a phase 2 study on heavily pre-treated patients with MGMT methylated metastatic colorectal cancer, temozolomide achieved about 30% of disease control rate. Activating mutations of RAS or BRAF genes as well as mismatch repair deficiency may represent mechanisms of resistance to alkylating agents, but a dose-dense schedule of temozolomide may potentially restore sensitivity in RAS-mutant patients. Further development of temozolomide in MGMT methylated colorectal cancer

Inno A, Fanetti G, Di Bartolomeo M, Gori S, Maggi C, Cirillo M, Iacovelli R, Nichetti F, Martinetti A, de Braud F, Bossi I, Pietrantonio F. Role of MGMT as biomarker in colorectal cancer. *World J Clin Cases* 2014; 2(12): 835-839 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i12/835.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i12.835>

ROLE OF MGMT IN THE DEVELOPMENT OF COLORECTAL CANCER

Colorectal carcinogenesis is a complex, multistep and still not completely understood process including both genetic and epigenetic alterations. DNA damage certainly plays a central role in cancer development and progression, especially when the DNA repair machinery is not

efficient.

O⁶-methylguanine DNA methyltransferase (MGMT) is a DNA repair enzyme codified by the *MGMT* gene at locus 10q26^[1]. MGMT removes alkyl groups from the O⁶-position of the guanine acting itself as an acceptor, and such reaction leads to an irreversible inactivation of the enzyme^[2]. *MGMT* transcription is regulated by epigenetic mechanisms. Methylation of the CpG dinucleotides in the promoter region of *MGMT* results in gene silencing, MGMT loss of expression and inability to remove alkyl groups from methylated guanine, with a consequent alteration of the normal DNA structure^[3].

While protecting normal cell from carcinogens, MGMT activity also protects tumor cells from lethal effects of chemotherapy with alkylating agents such as dacarbazine (DTIC) or temozolomide (TMZ), widely used for the treatment of melanoma and glioblastoma. In glioblastoma, in fact, *MGMT* methylation has been identified as a relevant prognostic factor and as an independent predictive factor of benefit from TMZ^[4,5]. For melanoma, the predictive and prognostic role of *MGMT* methylation status is controversial, but patients with MGMT-methylated melanoma treated with DTIC seem to be at higher risk of treatment-related adverse events^[6].

MGMT promoter methylation is a frequent and relevant event in colorectal cancerogenesis, with a low expression of MGMT secondary to gene silencing observed in 27 to 40% of metastatic chemorefractory colorectal cancer (mCRC)^[7]. However, MGMT loss has been also demonstrated in normal colorectal tissue, suggesting that *MGMT* silencing is only one of several steps needed for the accumulation of DNA damage and cell transformation. MGMT loss has been defined as a “field defect”, since it is neither necessary nor sufficient to cancer progression - *i.e.*, in a multistep process, it represents only one of the earlier passages leading to carcinogenesis. In fact, a second level of defense against DNA damage is represented by the mismatch repair (MMR) system which leads cell to apoptosis in presence of serious genomic alterations^[8]. Loss of MGMT expression is more frequent in CRC with microsatellite instability, suggesting that methylated *MGMT* select cellular clones with MMR deficient status^[9].

However, MGMT loss also plays a role in microsatellite-stable CRC through a mechanism of chromosomal instability^[10]. During DNA transcription, methylated guanine is wrongly recognized as adenine causing C:G to A:T transition mutations. MGMT may prevent guanine to adenine transition in ras-family genes, while the loss of MGMT activity may increase the likelihood of *RAS* and *TP53* mutations. In fact, *MGMT* methylation was found in 71% of mCRC with *KRAS* G>A mutations, whereas it was present only in 32% of tumors with non-G>A *KRAS* mutations and in 35% of tumors with wild-type *KRAS*^[11,12]. In *KRAS*-mutant tumors, *MGMT* promoter methylation occurs before *KRAS* mutations and it represents an early event in the adenoma-carcinoma sequence. Therefore, there should be a high concordance of *MGMT* methylation status between primary tumor and

distant metastases.

Despite its defined position in the pathogenesis of CRC, the prognostic role of *MGMT* is still controversial. Few studies directed to investigate the prognostic role of *MGMT* methylation have been published with different results. In a series of 116 patients, a reduction of recurrence rate after adjuvant therapy has been reported in *MGMT* methylated patients^[13]. Shima *et al*^[14] analyzed a group of 855 patients with CRC showing no prognostic value of MGMT loss or gene promoter hypermethylation. No benefit from 5-fluorouracil (5-FU)- or oxaliplatin-based regimens was reported in presence of *MGMT* promoter methylation or *MGMT* loss^[15].

ACTIVITY OF ALKYLATING AGENTS IN MGMT-METHYLATED COLORECTAL CANCER

DTIC and its oral derivative TMZ exert their cytotoxic effects through methylation of DNA at the N3 position of adenine and at the N7 and O6 positions of guanine. Although N7-methyl-guanine and N3-methyl-adenine represent the majority of adducts, cytotoxicity of DTIC and TMZ seems to be mainly due to DNA methylation at the O6-position of guanine, which leads to DNA double strand breaks and subsequent inhibition of DNA replication and apoptosis. As the cytotoxic effect of TMZ is mediated primarily through methylation of O6-guanine, the predominant mechanism of tumor resistance to DTIC and TMZ is *MGMT* expression.

TMZ showed *in vitro* activity against several human malignancies, including colorectal cancer^[16]. In a phase 1 study on solid tumors comparing a novel schedule for TMZ given twice a day for more than 5 consecutive days with the standard schedule of a daily administration for 5 consecutive days, the drug activity was quite disappointing with only 1 out of 12 mCRC patients responding to treatment^[17]. However, patients enrolled in the trial were not molecularly selected according to the *MGMT* methylation status.

In a pilot study, 66 patients with refractory metastatic cancer were treated according to the molecular tumor profiling, and TMZ was effective in 2 cases of advanced CRC exhibiting loss of *MGMT* expression^[18]. Consistently with these data, Schacham-Schmueli and colleagues described 2 patients with mCRC and a low expression of MGMT who were treated with TMZ achieving an impressive clinical response^[19]. To explore the hypothesis that in mCRC the activity of TMZ is confined to tumors with low levels of *MGMT*, a phase II trial combined the alkylating drug with lomeguatrib, a nontoxic low-molecular weight pseudosubstrate which inactivates MGMT^[20]. Unfortunately, the study was terminated early for futility, after 19 instead of the 30 patient initially planned had been recruited. The main reason why no objective response was observed, as the same authors stated, may be related to the low doses of TMZ and lomeguatrib used in the trial.

Table 1 Phase 2 clinical trials with alkylating agents in metastatic chemorefractory colorectal cancer

Ref.	Schedule	<i>n</i> (<i>MGMT</i> -m)	RR (<i>MGMT</i> -m)	DCR (<i>MGMT</i> -m)	PFS mo (<i>MGMT</i> -m)	OS mo (<i>MGMT</i> -m)
Amatu <i>et al</i> ^[21]	DTIC 250 mg/m ² per day, d 1-4 q21d	68 (26)	3% (8%)	12% (44%)	1.7 (NR) ¹	NR
Hochhauser <i>et al</i> ^[22]	TMZ 150 mg/m ² per day 7 d on/7 d off	37 ² (37)	3% (3%)	44% (445)	NR	NR
Pietrantonio <i>et al</i> ^[23]	TMZ 150 mg/m ² per day d 1-5, q28d	32 ³ (32)	12% (12%)	31% (31%)	1.8 (1.8)	8.4 (8.4)
Pietrantonio <i>et al</i> ^[26]	TMZ 75 mg/m ² per day, d 1-21 q28d	21 ⁴ (21)	24% (24%)	30% (30%)	2.2 (2.2)	NR

¹Median PFS for *MGMT*-m patients was not reported, but hazard ratio for progression between methylated and non-methylated patients was 0.66 (95%CI: 0.40-1.10), *P* = 0.0982; ²The study enrolled 86 patients with *MGMT*-m metastatic CRC (MCrc), esophageal, head and neck and non small cell lung cancer; this table reports data of patients with mCRC only; ³Only *MGMT*-m patients were enrolled in the study; ⁴Preliminary results. DTIC: Dacarbazine; TMZ: Temozolomide; RR: Response rate; DCR: Disease control rate; PFS: Progression-free survival; OS: Overall survival; *MGMT*-m: *MGMT*-methylated; NR: Not reported.

Amatu *et al*^[21] evaluated the activity of dacarbazine in 68 heavily pretreated mCRC patients. The response rate was only 3% with 2 partial responses, but a preplanned analysis based on *MGMT* methylation status in the individual tumors showed that objective responses only occurred in patients with *MGMT* methylated cancer. In the *MGMT*-methylated group, a significantly higher disease control rate (44% *vs* 6%, *P* = 0.012) and a trend toward longer progression-free survival (PFS) were also observed (Table 1). These results provided the proof-of-concept that dacarbazine, and consequently its derivative TMZ, are effective only in patients with mCRC harboring methylation in the *MGMT* gene promoter.

Patients with advanced aerodigestive tract and colorectal cancers and methylation of *MGMT* gene promoter were treated by Hochhauser *et al*^[22] with TMZ given at 150 mg/m² per day on a 7-day-on/7-day-off schedule. A low response rate (3%) was reported in patients with mCRC (Table 1), suggesting that *MGMT* methylation may be not the only factor determining response to TMZ. Particularly, cell death induced by TMZ also depends on the integrity of MMR pathways. In this study, all the patients with objective response were MMR-proficient, and most MMR-deficient patients experienced a disease progression, but data were limited to drive definitive conclusions about the correlation between MMR status and response to TMZ.

A phase 2 study run by our research group at the National Cancer Institute of Milan enrolled 32 patients with *MGMT*-methylated mCRC who progressed after all the approved standard therapies including fluoropyrimidines, oxaliplatin, irinotecan and, if *KRAS* wild-type, also cetuximab or panitumumab^[23]. All the patients received TMZ at the standard dose of 150 mg/m² per day for 5 consecutive days every 28 d until disease progression or unacceptable toxicity. This was the first trial of TMZ given to mCRC patients selected for *MGMT* methylation status. The study met its primary end-point of promising activity, with 4 (12%) partial responses and 6 (19%) disease stabilizations; median PFS and overall survival (OS) were 1.8 and 8.4 mo, respectively (Table 1). TMZ was well tolerated, with severe thromocytopenia occur-

ring in only 1 patient and no other grade 3-4 toxicities observed. Dose reduction was necessary in 3 patients, and no patients underwent early discontinuation due to adverse events. The study also explored potential predictive biomarkers, showing that none of the patients with mCRC harboring a mutation in the mitogen-activated protein kinases (MAPK), either *RAS* or *BRAF*, achieved a response. Conversely, four of nine patients with *RAS* and *BRAF* wild-type tumors had an objective response (0% *vs* 44%, *P* = 0.004). These results confirmed what was already shown for glioblastoma, namely that MAPK signaling may enhance *MGMT* activity and drive cellular resistance to TMZ^[24]. Interestingly, none of the patients included in this trial were MMR-deficient, and this might explain the clinically meaningful response rate observed in this study.

Other trials are currently ongoing, with the aim to shed more light on the role of TMZ as single agent and investigate predictive biomarkers in patients with *MGMT* methylated CRC (Eudract n. 2012-003338-17; 2012-002766-13).

FUTURE CHALLENGES

It was previously shown that a dose-dense TMZ regimen results in prolonged depletion of *MGMT* in blood mononuclear cells and possibly in the tumor^[25]. This schedule of administration may have enhanced activity due to higher cumulative dose and might be able to restore treatment sensitivity in *RAS* mutant tumors. Preliminary results from a mono-institutional, phase II, open label, single arm study with dose-dense TMZ were recently presented by our group^[26]. Patients with chemorefractory disease were treated with TMZ at a dose of 75 mg/m² given daily for 21 consecutive days of a 4-week cycle, up to 6 cycles or until disease progression or unacceptable toxicity. Seventeen out of 21 patients were evaluable for RECIST response. Tumor response, which was the primary endpoint of the study, was 24%. Interestingly, all patients with tumor response had a mutation of either *KRAS* (3 patients) or *BRAF* (1 patient). These preliminary data confirmed the encouraging activity of

TMZ in molecularly selected patients with *MGMT* methylation-positive, chemorefractory mCRC (Table 1). The good safety profile of dose-dense TMZ, as well as the response rate obtained in the *RAS* mutant population, are promising and warrant further prospective randomized confirmation.

There is a strong rationale for combining TMZ with fluoropyrimidines, based on preclinical data in slow-growing carcinoid cell line. It was found that TMZ and 5-FU have synergistic activity in a schedule-dependent manner in which 5-FU exposure preceded TMZ by 5–7 d with maximal synergism at 9 d. When the two agents were delivered concomitantly or if TMZ preceded 5-FU, *in vitro* cytotoxicity was additive but not synergistic. From these translational studies, researchers from the Columbia university formulated the CAPTEM regimen using TMZ for 5 d at 150–200 mg/m² per day refracted in a BID dosing on days 10–14 and capecitabine 750 mg/m² BID on days 1–14 of a 28-d-cycle^[27]. The TMZ was given twice-a-day instead of the standard daily dosing because the first dose binds *MGMT*, thus allowing the second dose to methylate guanines in presence of a decreased repair activity of *MGMT*^[17,28]. Given the potential synergy of CAPTEM combination in mCRC, we planned a randomized phase II study of second-line CAPTEM *vs* FOLFIRI after failure of prior first-line oxaliplatin-based treatment in patients with advanced, *MGMT* methylated, *RAS* mutated CRC (Eudract n 2014-002417-36). The primary objective of the study is to demonstrate the superiority of CAPTEM over standard FOLFIRI in terms of PFS; secondary endpoints are response rate, safety, quality of life, OS, with an exploratory biomarkers sub-study.

In conclusion, there is growing evidence that *MGMT* methylation status may serve as a predictive biomarker of response to TMZ in mCRC. TMZ is a promising agent for the treatment of *MGMT*-methylated mCRC, and the future research should establish the best schedule of TMZ and should also investigate how to integrate TMZ with the current available therapeutic options for mCRC, whether as single agent in chemorefractory patients or in combination with other active drugs in first or second line. The combination of TMZ with fluoropyrimidines is based on a strong rationale and is currently being investigated, but also the combination of TMZ with irinotecan^[29] has been proven to be feasible and it may deserve evaluation in mCRC. TMZ-containing chemotherapy may also provide an interesting backbone for the addition of biologic agents. However, the identification of additional biomarkers is a priority for future research, in order to select individual patients who may benefit the most from alkylating agents.

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Acute necrotizing pancreatitis: Surgical indications and technical procedures

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Abstract

Necrosis of pancreatic parenchyma or extrapancreatic tissues is present in 10%-20% of patients with acute pancreatitis, defining the necrotizing presentation frequently associated with high morbidity and mortality rates. During the initial phase of acute necrotizing pancreatitis the most important pillars of medical treatment are fluid resuscitation, early enteral nutrition, endoscopic retrograde colangiopancreatography if associated cholangitis and intensive care unit support. When infection of pancreatic or extrapancreatic necrosis occurs, surgical approach constitutes the most accepted therapeutic option. In this context, we have recently assisted to changes in time for surgery (delaying the indication if possible to around 4 wk to deal with "walled-off" necrosis) and type of access for necrosectomy: from a classical open approach (with closure over large-bore drains for continued postoperative lavage or semiopen techniques with scheduled relaparotomies), trends have changed to a "step-up" philosophy with initial percutaneous drainage and posterior minimally invasive or endoscopic access to the retroperitoneal cavity for necrosectomy if no improvement has been previously achieved. These approaches are progres-

sively gaining popularity and morbidity and mortality rates have decreased significantly. Therefore, a staged, multidisciplinary, step-up approach with minimally invasive or endoscopic access for necrosectomy is widely accepted nowadays for management of pancreatic necrosis.

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Key words: Acute pancreatitis; Necrotizing pancreatitis; Surgery; Open necrosectomy; Minimal access retroperitoneal pancreatic necrosectomy; Video-assisted retroperitoneal debridement

Core tip: We have recently assisted to a significant change in surgical approach to acute pancreatitis. Infection continues as the most important pillar where surgical indication is established. Nevertheless, from an early consideration for surgery frequently performed by classical open approach, today we have moved to delay the indication and the procedure as much as possible with step-up philosophies trying to deal with "walled-off" necrosis and considering minimally invasive access like video-assisted retroperitoneal or endoscopic. In this paper, most recent therapeutic trends for acute necrotizing pancreatitis are reviewed.

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INTRODUCTION

Gallstones and alcohol are still the most frequent causes of Acute Pancreatitis (AP), a disease with an increasing

Table 1 Revised definitions of acute pancreatitis (2012)^[1,2]

Types	
Interstitial edematous	Inflammation of pancreatic parenchyma and peripancreatic tissue, without necrosis
Necrotizing	Inflammation with pancreatic parenchymal and/or peripancreatic necrosis
Grades of severity	
Mild	No organ failure
Moderate	Lack of local or systemic complications
	Transient organ failure (< 48 h) and/or Local or systemic complications without persistent organ failure
Severe	Persistent single or multiple organ failure (> 48 h)

incidence in recent decades. Diagnosis is based on the presence of two of the following criteria: (1) characteristic upper abdominal pain radiating in a belt-shaped fashion; (2) amylase or lipase values three times above normal levels; and (3) demonstrative radiologic imaging.

Due to the confusion created by certain terminology derived from the Atlanta classification of AP, a recent revision of the terminology has been developed from an International Consensus, which currently constitutes the reference of the conceptual definition of AP (Table 1). AP is classified into its two forms of presentation (Interstitial Edematous Pancreatitis and Necrotizing Pancreatitis), and according to the severity of the clinical course (mild, moderate and severe). Most AP episodes occur as an interstitial edematous form (80%-90%), usually associated to a mild clinical course, while more clinical severity is frequently associated to the characteristic that defines acute necrotizing pancreatitis (ANP): necrosis, pancreatic or peripancreatic, or a mix of both^[1,2].

Local complications associated to the edematous form are defined depending on whether they have or not a well-defined wall and on the time of their arising. Acute fluid collections arise within the first 4 wk of the clinical course and lack a well-defined wall, while pseudocysts are circumscribed fluid collections that occur more than 4 wk after the onset of AP and have a well-defined wall. These complications are out of the scope of the current revision, since their surgical management is not necessary or it is included among elective treatments. On the other part, necrotizing forms may be associated to acute necrotic collections (intra- or extra-pancreatic solid-liquid heterogeneous collection with no defined wall, diagnosed during the first 4 wk of the clinical course) or walled-off necrosis (with similar characteristics but with well-defined wall and with a later diagnosis, above 4 wk). This later concept gathers all previous terms (necroma, pancreatic sequestrum, subacute pancreatic necrosis or pancreatic pseudocyst with necrosis) into a common terminology. Other local complications associated to AP include splenic/portal thrombosis, colon necrosis, retroperitoneal hemorrhage or delayed gastric emptying^[1,2].

Physiopathologically, two events may determine the severity of the clinical course. The first of them is the associated Systemic Inflammatory Response Syndrome

(SIRS), which involves a complex inflammatory cascade, which can finally cause the characteristic single- or multiorgan failure. Respiratory, renal and cardiovascular are the most frequent organic failures associated to AP. The second event is infection of necrosis, which is usually associated to phenomena of bacteria translocation. Both events constitute critical factors determining the clinical course of AP and they establish even the current indications for surgery in AP; thus, they will be expounded in detail later on^[3].

SURGICAL IMPLICATIONS DURING THE FIRST WEEK

During the first week after the onset of AP, treatment is medical. Frequently, surgeons on duty are called to evaluate patients with AP without response to medical treatment during the initial phase of the disease. For decision making in this context it must be assumed that the reason for lack of response in these patients is based more in the presence and progression of SIRS than in the potential necrosis or pancreatic infection. Thus, surgery is not indicated during this phase, unless a suspicion of ischemia or perforation as a secondary complication arises. Surgery during this first phase aggravates the multiorgan failure and results in a greater rate of complications, such as intestinal hemorrhage or fistula^[4].

In this phase, fluid resuscitation is essential during the first 12-24 h, having to be reduced later on trying to avoid intra abdominal hypertension (IAH), frequently associated to AP. A recent review could not find any difference between fluid resuscitation with colloid or crystalloid solutions^[5]. Prophylactic antibiotics are not indicated, since they have not shown a clear benefit in previous studies and metaanalysis, and thus, they should not be used until an associated infection is clearly demonstrated^[4]. Early (on the first 72 h) Endoscopic Retrograde Cholangiopancreatography has not shown benefits when performed systematically in AP in the absence of cholangitis, although an ongoing clinical trial is analyzing this approach again (APEC trial; ISRCTN97372133). However, in presence of bile duct obstruction it is clearly indicated^[6].

During this first week, surgeons are occasionally requested to evaluate patients with AP and IAH, present in 61% of episodes of AP. IAH is the precursor of the Abdominal Compartment Syndrome (ACS) and, thus, of multiorgan failure. Although the beneficial effect of decompression to alleviate ACS is clear in other situations, in AP the ACS seems to be closer related to massive resuscitation, ascites or retroperitoneal fluid accumulation and, thus, treatment strategies with decompressive laparotomy have not shown a clear benefit in terms of morbidity and mortality. Currently, management strategies for AP-associated ACS are directed towards medical support (negative fluid balance, enteral decompression, pharmacological increase of the abdominal wall compliance) and even towards percutaneous drainage of fluid collections (with a related ongoing clinical trial, the DECOMPRESS

Table 2 Modified Marshall scoring system for organ or multiorgan failure^[2,7]

Organ system	0	1	2	3	4
Respiratory (PaO ₂ /FiO ₂)	> 400	300-400	200-300	100-200	< 100
Renal (Creatinine, mg/dL)	< 1.4	1.4-1.8	1.8-3.6	3.6-5	> 5
Cardiovascular (mmHg)	> 90	< 90, fluid responsive	< 90, not fluid responsive	< 90, pH < 7.3	< 90, pH < 7.2

Study; ClinicalTrials.gov NCT00793715). In case abdominal surgical decompression had to be performed during this first phase, in absence of infected necrosis, the retroperitoneum should not be opened^[4].

SURGICAL IMPLICATIONS AFTER THE FIRST WEEK

This is the moment to consider the necessity and indication for surgery of the pancreatic necrosis, on which we will expound extensively. Other local complications may occur at this phase but they are much less frequent and will be mentioned at the end of the chapter.

SURGERY FOR PANCREATIC NECROSIS

Indications

Most common indications for surgery of pancreatic necrosis are the following: (1) infection. It is a rare event during the first week of clinical course. The diagnosis is based on the association of sepsis signs with compatible radiologic imaging (extraluminal air in intra- or extra-pancreatic necrotic areas in CT imaging) and on the occasional support of vascular radiologists with percutaneous fine-needle aspiration for Gram staining and culture. There is universal consensus that a need for therapeutic action exists; (2) single- or multiorgan failure. Organ failure is classified as transient or persistent based on whether it lasts less or more than 48 h, respectively. The most recommended system for its definition (even above the Sepsis-related Organ Failure Assessment -SOFA-) is the Marshall score^[7] (Table 2), which is easy and repeatable along the clinical course of AP. It evaluates the three most commonly SIRS-affected systems (respiratory, renal and cardiovascular) and defines organ failure as a score of 2 or more.

A persistent single- or multiorgan failure refractory to support treatment may constitute an indication for surgery. Numerous studies have shown that in this context, oppositely to what happens when infection constitutes the indication for surgery, necrosectomy does not provide a significant benefit regarding mortality, and thus, it must be considered as the last resource in a patient in whom maximum medical support does not result in clear improvement. We can assert the same statements when surgical indication is established on a patient with ANP with no clinical improvement after 4-6 wk of intensive medical treatment.

We must consider that the indication for surgery in the context of AP must derive more from the need to control complications than from the inflammatory pro-

cess itself. Regarding this, every necrotic and infected tissue must be removed, and pus drained. Material viscosity, as well as the number and localizations of potentially drainable regions constitute determining factors for the selection of the best therapeutic approach. Morbidity associated to pancreatic debridement includes pancreatic fistula (50%), endo- and exocrine pancreatic failure (20%), intestinal fistula (10%) and the common prolonged hospitalization and delay in the incorporation to daily life activities^[3,4].

It is important to underline some important concepts before describing the different surgical options: (1) debridement is preferred over resection for two reasons: first, as an attempt to conserve the maximum quantity of functional pancreatic tissue, and second, due to the frequent technical impossibility of pancreatic resection and its associated morbidity in the context of AP^[3,4]; (2) unless evident infection of necrosis exists, survival improves as the surgical indication gets delayed. The best results are obtained when the indication may be delayed up to one month after the onset of the clinical symptoms. A better demarcation of necrosis (conversion to “walled-off” pancreatic necrosis) involves less bleeding and less removal of viable tissues^[3,4]; and (3) Two different philosophies define the timing of the surgical approach for a patient with AP. “Step-down” consists on the classical immediate surgical approach when there is an established indication, and, later on, a more conservative treatment for the residual disease. However, there is a trend in the most recent medical literature towards a “step-up” type of concept, where more conservative procedures (percutaneous, laparoscopic or endoscopic procedures) constitute the initial treatment of patients with ANP and a final technique is performed later on if necessary, based on poor clinical evolution^[8].

Options

Open necrosectomy: Open necrosectomy (ON) was considered as the gold standard treatment for decades, and it was usually associated to a therapeutic “step-down” type of approach. Classical ON consists on debridement of the necrotic pancreatic tissue through a midline or subcostal bilateral incision and the access to the pancreatic area through the lesser sac, the gastrocolic omentum or by a transmesenteric access through the transverse mesocolon, depending on necrosis extension and localization. Once the necrosectomy has been performed, the options are: (1) usual closure over drains and relaparotomy depending on clinical course; (2) scheduled laparotomies, usually every 48 h, until debridement has been completed. Open abdomen techniques are recommended if this

approach is selected, but scheduled laparotomies closing the abdomen after each revision have also been reported; and (3) closed technique with abdominal closure over lavage system with large-bore drains in the pancreatic area.

The last one constitutes the most recommended option based on a mortality < 10%, significantly inferior to those associated to the rest of the techniques. Nevertheless, an effective comparison between the different methods is difficult because of the heterogeneity of patients and surgeons^[3,4,9].

Percutaneous drainage: Several series of patients have reported that management of infected pancreatic necrosis with percutaneous drainage (PD) obtains a high success rate and mortality similar to that of ON treatment. In a systematic review^[10], the success rate of PD (defined as survival with no need for additional surgical necrosectomy) was 55%, mortality 17% and morbidity 21%, showing pancreaticocutaneous and pancreaticoenteric fistulas as the most frequent complications associated to the procedure. Although PD constitutes a tempting and efficient therapeutic alternative as a minimally aggressive approach, the truth is that frequently success depends on the availability of large caliber catheters and often repeated procedures are needed. For selected patients, like those with AP and easily accessible single infected necrosis, or as a transient step to surgery in unstable patients, this therapeutic option must be considered. However, PD is not accepted as a useful tool when an extensive necrosectomy is needed^[3,4,9].

Endoscopic approach: A promising approach for pancreatic necrosis is the transgastric or transduodenal endoscopic approach (EA) under direct vision or with ultrasound support. Very diverse types of instruments are later used to maintain opened the communication between the digestive lumen and the necrosis and to perform the necrosectomy, and frequently repeated procedures are needed. Several series have reported promising results with EA, some as important as the German multicenter GEPARD Study^[11] with 93 patients, 81% of clinical success and only 7.5% mortality. The results of EA for necrotizing AP have been recently summarized in a systematic review^[12], which reports a success rate of 75% on 260 patients; however, it must be pointed out that data derive from non-randomized studies on selected patients in reference centers.

Laparoscopic approach: Laparoscopic approach (LA) for ANP may result particularly attractive because of its potential of obtaining all the advantages of a minimally invasive approach while maintaining access to the whole abdominal cavity (perirenal and retroduodenal spaces and mesenteric root, as well as both paracolic gutters) and the technical possibility of indicating additional techniques (cholecystectomy or jejunostomy). However, the extension of LA in ANP among professionals is still scarce, since its advantages are overpassed by its limitations: infection dissemination, need for pneumoperitoneum in

unstable patients, or the possibility of iatrogenic intestinal perforations. Published series of patients have a scarce number of cases and it is still soon to recommend this approach^[3,4].

Retroperitoneal approach: It constitutes the maximum example of Minimally Invasive Necrosectomy. Retroperitoneal approach (RA) of the necrosis is performed through small incisions and the use of endoscopic material, guided by a percutaneous drainage previously and strategically indicated, placed laterally, avoiding access to the abdominal cavity and providing all the advantages of the minimally invasive approach. Diverse methods for its performance have been described but the most widely accepted are Minimal Access Retroperitoneal Pancreatic Necrosectomy (MARPN) and Video-Assisted Retroperitoneal Debridement (VARD) (Figure 1). This last one consists in the introduction of a laparoscopic camera, through a 5 cm incision, and after the first liquid and solid debris have been removed a vigorous debridement of the necrotic cavity may be performed with the maintenance of a low-pressure pneumoperitoneum. The number of repeated necrosectomies needed later on is significantly lower with VARD than with MARPN.

In a systematic review, incidence of multiorgan failure, incisional hernia and endo- and exocrine failure was significantly lower with RA than with ON, although mortality was similar and no differences were observed regarding local complications, such as intraabdominal bleeding or pancreatic fistula^[13].

Combined approaches with “step-up” philosophy: Probably, management of pancreatic necrosis in an immediate future will not be based only in one of the methods already mentioned. A combination of them with a decision making based on characteristics of the patient, necrosis grade, extension and localization will be the key for defining the best therapeutic option in ANP. The most illustrative of these approaches is the Dutch PANT-ER^[14] (PANcreatitis, Necrosectomy *vs* sTEp up appRoach) study, a randomized, multicenter, clinical trial in which 88 patients with necrotizing AP who met inclusion criteria, and in whom the need to perform an invasive procedure was established (delayed whenever as possible up to 4 wk from the onset of the clinical symptoms) were divided into two groups: 45 with ON and 43 with “step-up” type minimally invasive approach. Such an approach consisted in placing a percutaneous drainage as the first step (40 retroperitoneal, 1 abdominal and 2 endoscopic), assessing the placement of a second drainage or the performance of RA in the absence of improvement after 72 h, based on clinical and radiologic findings. The morbi-mortality rate of the “step-up” type of approach was significantly lower than that of the ON group (40% *vs* 69%, $P < 0.006$), and so was the multiorgan failure (12% *vs* 40%, $P < 0.002$). In addition, it was defined that more than 30% of patients in the “step-up” group did not need necrosectomy after the percutaneous or endoscopic drainage.

On the same line of therapeutic combination, the on-

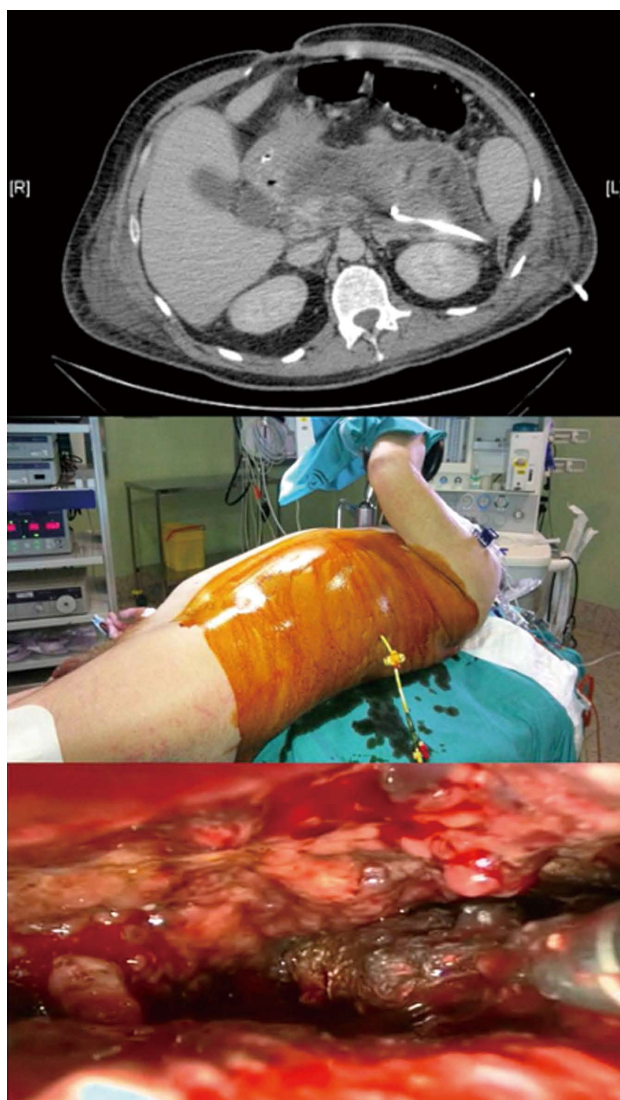


Figure 1 Video-assisted retroperitoneal debridement: Computed tomography scan with percutaneous drainage into an infected pancreatic necrosis (above); patient's position for necrosectomy (middle); endoscopic view of necrotic pancreatic tissue (below).

going TENSION study (ISRCTN 09186711) randomizes 98 patients with ANP to receive step-up type endoscopic approach vs surgical approach.

OTHER SURGICAL INDICATIONS

Disruptions of the main pancreatic duct may derive in internal or external fistulas, pancreatic ascites or pleural effusion. Treatment constitutes an important challenge and, depending on location and clinical manifestation, it can require from PD as the only procedure up to pancreatic resection or Roux-en-Y derivation for what is called the Disconnected Pancreatic Duct Syndrome, although a CPRE approach and transpapillary drainage is usually sufficient^[3].

Vascular complications occur in 2.4%-10% of patients with AP, and they derive from bleeding into the peritoneal cavity or into the gastrointestinal tract from pseudoaneurysms of vessels close to the inflammatory

process, such as the gastroduodenal or pancreaticoduodenal arteries. Nowadays, embolization is the therapeutic method of choice^[3,4].

Colonic complications associated with pancreatitis are infrequent (1% of cases). From reactive ileus to most severe forms with obstruction, necrosis or perforation are associated to poor prognosis and mortality increase. For the majority of cases, resection with proximal ostomy and mucous fistula constitutes the treatment for these complications.

Maybe out of the emergency context but of necessary mention, it must be underlined that there is an indication for cholecystectomy in the first admission after the first mild gallstones pancreatitis. A systematic review reported up to 18% readmissions in patients with mild AP if an early cholecystectomy is not indicated and performed^[15]. In severe AP, however, cholecystectomy should be deferred until complete resolution of inflammation^[16].

CONCLUSION

In recent years we have witnessed a change in the management of patients with ANP: from a prematurely indicated surgery, performed open, we are moving to less aggressive procedures, indicated later and with "step-up" strategies, performing first PD, preferably retroperitoneal, and continuing with surgical (considering new access routes) or endoscopic necrosectomy in case of absence of improvement. These concepts require a multidisciplinary approach to ANP with implication of surgeons, gastroenterologists, radiologists, and intensive care unit doctors, and the need for contemplating the referral of patients to reference centers when the needed logistic is not available. Trauma and Emergency Surgery Units in the Departments of General and Digestive Surgery constitute, thus, the ideal setting for the early diagnosis, indication, intervention and follow-up of these patients.

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Large bowel injuries during gynecological laparoscopy

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Abstract

Laparoscopy is one of the most frequently preferred surgical options in gynecological surgery and has advantages over laparotomy, including smaller surgical scars, faster recovery, less pain and earlier return of bowel functions. Generally, it is also accepted as safe and effective and patients tolerate it well. However, it is still an intra-abdominal procedure and has the similar potential risks of laparotomy, including injury of a vital structure, bleeding and infection. Besides the well-known risks of open surgery, laparoscopy also has its own unique risks related to abdominal access methods, pneumoperitoneum created to provide adequate operative space and the energy modalities used during the procedures. Bowel, bladder or major blood vessel injuries and passage of gas into the intravascular space may result from laparoscopic surgical technique. In addition, the risks of aspiration, respiratory dysfunction and cardiovascular dysfunction increase during laparoscopy. Large bowel injuries during laparoscopy are serious complications because 50% of bowel injuries and 60% of visceral injuries are undiagnosed at the time of primary surgery. A missed or delayed diagnosis increases the risk of bowel perforation and consequently

sepsis and even death. In this paper, we aim to focus on large bowel injuries that happen during gynecological laparoscopy and review their diagnostic and management options.

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Key words: Colon; Gynecology; Intraoperative complications; Laparoscopy; Wounds and injuries

Core tip: Large bowel injury during laparoscopy is a serious complication because 50% and 66% of bowel and visceral injuries are undiagnosed at the time of primary surgery. A missed or delayed diagnosis increases the risk of bowel perforation and consequently sepsis and death.

Ülker K, Anuk T, Bozkurt M, Karasu Y. Large bowel injuries during gynecological laparoscopy. *World J Clin Cases* 2014; 2(12): 846-851 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i12/846.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i12.846>

INTRODUCTION

Four decades ago, laparoscopic surgery was being performed by a limited number of surgeons and most of the procedures were limited to diagnostic laparoscopy and tubal sterilization. However, through the years laparoscopy has evolved and become one of the major management choices for many surgical diseases. Cameras and hand instruments with improved visual quality and better manipulation capabilities, respectively, along with the accumulation of the data obtained from previous studies and case reports have contributed to the evolvement of laparoscopy.

Today, laparoscopy is one of the most frequently preferred surgical options in gynecological surgery. In the United States, roughly 350000 bilateral tubal sterilizations and 200000 hysterectomies are performed using

laparoscopy each year. The popularity of laparoscopy has increased around the world and many gynecologists, including inexperienced and junior surgeons in training, have begun to perform laparoscopic procedures. Thus, the number of patients prone to complications during laparoscopy has increased^[1].

Laparoscopy has advantages over laparotomy, including smaller surgical scars, faster recovery from surgery, less pain and earlier return of bowel functions. Generally, it is also accepted as safe and effective and patients tolerate it well^[2]. However, it is still an intra-abdominal procedure and has the similar potential risks of laparotomy, including injury of a vital structure, bleeding and infection^[3-6]. Intra and postoperative complications are below 1% and 4 to 8 patients are lost per 100000 laparoscopic procedures.

Besides the well-known risks of open surgery, laparoscopy also has its own unique risks related to abdominal access methods, pneumoperitoneum created to provide adequate operative space and the energy modalities used during the procedures. Bowel, bladder or major blood vessel injuries and passage of gas into the intravascular space may result from laparoscopic surgical technique. In addition, the risks of aspiration, respiratory dysfunction and cardiovascular dysfunction increase during laparoscopy^[5-9]. Blood loss is generally lower than in open surgery; however, in some cases, massive blood loss necessitates immediate laparotomy.

Because of its advantages over laparotomy, such as less pain, hospital stay and recovery time, laparoscopy is generally perceived as a minor surgical procedure by patients. Thus, the medico legal aspects of the complications of laparoscopy are prone to exaggeration. In order to minimize complications and their unavoidable consequences, surgeons should learn the probable complications and their management. In this paper, we aim to focus on large bowel injuries that happen during gynecological laparoscopy and review their diagnostic and management options.

CLASSIFICATION, EPIDEMIOLOGY AND RISK FACTORS

Complications related to laparoscopic surgery can occur during either intra or postoperative phases. Intraoperative complications can further be divided into complications of access and complications of the operative procedure. More than half of complications occur at the setting up phase, particularly during the creation of the abdominal access pathways necessary for the telescope and trocars^[10,11].

The complication rate during the placement of the initial abdominal access port is less than 1%. Complications following the initial access are also rare. In contrast, port site hernia as a late complication can affect 6% of patients^[12-14]. Although rare, severe complications including vascular and bowel injuries, may cause serious morbidity and even result in the death of the patient.

The study conducted by Chandler *et al*^[10] in 2001 showed that the incidence of injury during abdominal access varied between 5 and 30 per 10000 procedures. Large bowel was the third most frequent injury site after the small bowel and iliac artery, with 12% of all injuries at the large bowel. In their review published in 2012, Jansen *et al*^[15] reported that access related bowel injury was seen in 4.4 per 10000 gynecological procedures. In addition, Hasson's open abdominal access technique did not significantly lower the complication rates compared to the closed technique.

Bhoirul *et al*^[16] studied 32 deaths following 629 trocar injuries and found that six patients died following bowel injury. Delay in the diagnosis of gastrointestinal perforation resulted in a mortality rate of 21%.

A history of previous intra-abdominal surgery, vertical incision, endometriosis and pelvic infection increases the risk of bowel injury. Extensive bowel distension obscuring the operative field, large abdominal or pelvic mass (in the case of hysterectomy, uterine size over 500 g) and diaphragmatic hernia increase the risk of complications. In addition, major operative laparoscopy, extensive adhesiolysis and concomitant major surgery are the other factors that increase the risk of complications. Moreover, surgeon experience and the type and the difficulty of the cases also contribute to complication rates^[17-21].

LARGE BOWEL INJURIES DURING THE SETTING UP PHASE OF GYNECOLOGICAL LAPAROSCOPY

Bowel injury may be encountered at any stage of laparoscopic surgery, beginning from abdominal access until the end of port site closure. It is the third most frequent mortal complication of laparoscopy, following anesthesia and major vessel injuries^[22]. Gastrointestinal tract injury during laparoscopy ranged between 0.03 and 0.18%^[6,23-26] and its incidence was 0.13% in the meta-analysis performed by van der Voort *et al*^[27].

Before the study performed by Levy *et al*^[28], energy modalities used in laparoscopic surgery were mistakenly considered to be the leading cause of gastrointestinal injuries. However, 30% to 50% of the bowel injuries occur during Veress needle or trocar insertion into the abdominal cavity^[6,11,29-31]. Gastrointestinal injuries occur more often at the small bowel; however, other intra-abdominal organs, including the large bowel and stomach, may also be injured. Preoperative bowel preparation and decompression of the stomach with an orogastric or nasogastric tube may prevent potential injuries occurring during abdominal access.

In the retrospective case review study conducted by Chapron *et al*^[5], 32.1% of the gastrointestinal injuries occurred during the initial set up procedure. Pneumoperitoneum needle, umbilical trocar and suprapubic trocar were isolated as the causes of injuries in 10.7%, 16.1% and 5.3% of the cases, respectively. Of the 62 gastrointestinal injuries of the 56 patients, 57.2% occurred during the

operative phase of the procedure, and electrosurgery and sharp dissection were the causes of injuries with the rates of 10.7% and 46.5%, respectively. The authors could not define whether the injuries occurred during initial set up or operative phases in 10.7% of the cases.

Of the 62 gastrointestinal injuries of the 56 patients reviewed by Chapron *et al*^[5], 30 (48.4%) injuries involving the large intestine had the highest frequency and were followed by the 21 (33.9%) small bowel and 10 (16.1%) epiploon injuries. Of the 30 large bowel injuries, 18 injuries were at the sigmoid colon, followed by four cecum, four rectum and four colon injuries.

It is not clear whether the frequency of bowel injury during abdominal access is affected by the complexity of the operative phase. Some studies reported higher rates of bowel injury during access in diagnostic laparoscopy and laparoscopic tubal sterilizations^[30], in contrast to others reporting higher injury rates in major laparoscopic surgeries^[6].

The surgeon's experience affects the rate of injury; however, the frequency of injuries during abdominal access is still high for more experienced surgeons^[32]. Depending on the fact, investigators are trying to improve the outcomes of abdominal access during laparoscopy by using various access techniques. Blind Veress introduction followed by pneumoperitoneum and the primary trocar, direct trocar insertion and open access techniques are examples. In addition, investigators are trying to improve the already known techniques. As an example, in their recently published study, Ozdemir *et al*^[33] used umbilical stalk elevation (USE) technique to improve the success rate of Veress needle insertion in obese patients and concluded that the USE technique seemed safe and required a significantly fewer number of attempts to create pneumoperitoneum.

Excellency in Veress needle and trocar use may prevent some major complications. Although wiggling of the needle movements to ascertain intra-abdominal entry may enlarge the diameter of an injury^[32], the correct placement of the needle is usually checked by most surgeons. In addition to the classical safety checks, foul smell, observation of the gastrointestinal contents and asymmetrical abdominal distention due to insufflation of the bowel should raise the suspicion of bowel injury^[34]. Moreover, passage of flatus may be a sign of intra-intestinal insufflation.

Although Hasson's open technique did not lower the total complication rates, theoretically open techniques may decrease the risk of life threatening major vascular injuries during abdominal access. In addition, the chance of an earlier diagnosis is higher. In contrast to the theoretical advantages of an open technique, there are articles reporting a higher incidence of bowel injury with an open technique^[29,35,36]. However, many surgeons prefer open access techniques for patients with anticipated risks. Thus, in order to avoid selection bias, final judgment will be appropriate after randomized prospective studies.

In addition to their theoretical advantages, open techniques are also used during gasless laparoscopies and may help in lowering the CO₂ related risks of laparo-

scopic surgery. Thus, gasless laparoscopy may decrease some risks of laparoscopic surgery that occur during abdominal access. In our practice, we have experienced the single incision, gasless technique called keyless abdominal rope-lifting surgery (KARS)^[37-40] and did not observe any internal organ injury. However, among the various access techniques, the best probably is the one in which the surgeon has more experience and advanced skills.

LARGE BOWEL INJURIES DURING THE OPERATIVE PHASE OF GYNECOLOGICAL LAPAROSCOPY

During the operative phase of laparoscopy, bowel injury may occur as a result of trauma secondary to tissue dissection and manipulation or electrosurgical energy use. It is a serious complication because 50% and 66% of bowel or visceral injuries are undiagnosed at the time of primary surgery^[41]. A missed or delayed diagnosis increases the risk of bowel perforation and consequently sepsis, and even death^[6].

In the study conducted by Chapron *et al*^[5], of the 56 patients suffering from gastrointestinal injury, 32 had injuries at the operative phase of the procedures and 26 injuries were due to sharp dissections. Thus, experienced surgeons with advanced surgical skills are expected to have lower complication rates. Not surprisingly, experience significantly decreases the complication rates of the operative phase and the surgeon's advanced skills in fine adhesiolysis also decreases the complication rates^[6].

Brummer *et al*^[42] compared the incidence of injuries of laparoscopy performed between 1992 and 1999 with the injury incidence of 2000 and 2005, emphasizing the importance of the learning curve in laparoscopic and vaginal hysterectomies. The incidence of all kinds of injuries was significantly lower between 2000 and 2005. Similarly, bowel injuries during laparoscopic hysterectomies decreased from 0.14% to 0.09% during the same period and large bowel injuries involved half of all bowel injuries^[42]. The use of proper hand instruments while manipulating and dissecting the tissues may decrease the injury rates.

The use of electrosurgical energy during operative laparoscopy causes injury of the target tissue. The injured tissue may become necrotic or heal slowly during the postoperative period^[43]. In addition to the target tissue, increased local temperature may cause injury of the nearby vital structures, *e.g.*, the large bowel. Thus, the surgeon should be familiar with the used energy modality. A monopolar current travels through the tissues of the patient; however, a bipolar current passes between the two electrodes of the instrument and thus influences only the tissue between electrodes.

Monopolar energy causes more lateral thermal spread and produces the highest temperatures compared to bipolar electrocautery, the Harmonic scalpel and LigaSure^[44]. The degree of lateral thermal spread varies with various energy modalities and is as follows: 2-22 mm for

traditional bipolar, 0-3 mm for ultrasonic cutting and coagulation, 1.1 mm for the Enseal, 1.8 mm for LigaSure and 6.3 mm for Gyrus Plasma Trisector^[45-48]. In addition, the monopolar electrosurgical instrument insulating layer is not foolproof and the current may spread to the adjacent tissue^[49]. Thus, in a case where the operative field is close to the bowel, the risk of bowel injury increases and the unnoticed injury may present postoperatively.

PREVENTION, DIAGNOSIS AND MANAGEMENT

Most gynecologists learn traditional gynecological procedures during residency; however, they generally gain skills required for laparoscopic procedures during their postgraduate clinical practice without supervision. The learning curve is lengthy and becomes longer with the advancement of new techniques and instruments. The complication risk is highest during the initial stages of a surgeon's laparoscopic experience^[50].

A comprehensive preoperative evaluation, proper consultations, patient selection and risk assessment help lessen the risk of complications. Besides a gynecologist having the required skills for laparoscopic surgery, the operating room staff and assistants should also be properly trained. The operating room should be ready for an emergency laparotomy. The infrastructure required for a multidisciplinary surgical approach should be maintained during the laparoscopic procedures.

During the initial stages of the experience of laparoscopy, it is better for a surgeon to perform minor procedures. Previous studies reported that the complication rates were higher in the first 100 procedures of surgeons beginning to perform laparoscopy.

Sudden and uncontrolled Veress needle and trocar entry can lacerate the rectum and sigmoid colon. The transverse colon may be displaced by the distended stomach and become vulnerable to injuries. A nasogastric tube helps to eliminate this potential risk.

Obliteration of the pouch of Douglas and the presence of dense adhesions between the rectum and uterus increase the chance of bowel injury. In these circumstances, blunt dissection may increase the chance of rectal laceration and thus sharp dissection with scissors or CO₂ laser should be preferred. Placement of a probe or finger in both the vagina and the rectum helps to identify the tissue planes. Nezhat *et al.*^[51] and Redwine^[52] advise beginning the dissection lateral to the uterosacral ligaments and proceeding toward the obliterated cul-de-sac. In addition, preoperative bowel preparation may help in cases with high risks for bowel injury.

One to two thirds of bowel injuries can be detected intraoperatively^[5] and half of the injuries can be identified between first and seventh postoperative days. Most patients do not have the typical symptoms of bowel injury, such as low-grade fever, nausea, vomiting, ileus, severe abdominal pain, leucopenia or a normal leukocyte count, and the diagnosis is delayed. Thus, in many cases, patients

present with peritonitis and the situation increases the rates of morbidity and mortality^[10,16]. Sepsis and acute abdominal pain are typically observed 1-2 d after surgery.

Brownish fluid in a saline aspiration test may sometimes diagnose large bowel perforation. In addition, fecal smell strengthens the suspicion. In cases where the suspicion of bowel perforation arises, the Veress needle should be replaced with a sterile one and the field beneath the primary entrance should be examined after the introduction of the telescope. Intraoperative sigmoidoscopy may be helpful in identifying the injury site^[53]. Recently, in an experimental study conducted by Ülker *et al.*^[54], insertion of a rectal catheter attached to a urine bag was recommended to identify large bowel injuries. It was suggested that the accumulation of gas in the connected bag would signal small and hardly demonstrable large bowel injuries. A computerized tomography examination can reveal fecal material outside the large bowel and/or free air in the abdominal viscera. Additional imaging work up, including imaging with a gastrografin enema, can also help to detect an injury site.

Large bowel injuries should be managed at the time when they are recognized, if possible, at the same operative section. Small injuries secondary to a Veress needle may be managed conservatively with close observation in hospital, intravenous hyperalimentation and antibiotics^[55]. However, 6% of cases with superficial electrocautery bowel injuries require open exploration due to acute perforation during the observation period and thus intraoperative repair of the damaged bowel is significantly safer and should be performed in every suspicious electrocautery bowel injury.

Most trocar injuries need a primary closure in one or two layers. However, larger injuries with an ambiguous tissue injury may necessitate colostomy. In these conditions, incorporation of a general surgeon experienced with bowel surgery is advisable. Depending on the skills of the surgical team, bowel repair may be performed laparoscopically^[56]. Extensive intra-abdominal lavage, use of combined broad-spectrum antibiotics and drainage may decrease the infection risk.

Injury at the right ascending colon generally requires resection of the injured section and a primary anastomosis. Ileostomy with diversion of the intestinal contents speeds up healing. In a case where the bowel is not prepared preoperatively and the descending colon, sigmoid or rectum is injured, primary closure or resection with primary anastomosis are not good treatment options. In these circumstances, a diverting colostomy with resection of the injured portion is recommended. Colonic lacerations of preoperatively prepared bowel can be repaired laparoscopically^[50].

CONCLUSION

Large bowel injuries during gynecological laparoscopy are rare but serious complications. Approximately one third can be diagnosed intraoperatively and delayed diagnosis increases the rates of morbidity and mortality. They

should be managed immediately when recognized, if possible, at the same operative section.

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Aetiology of idiopathic granulomatous mastitis

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Abstract

Idiopathic granulomatous mastitis is a rare chronic inflammatory lesion of the breast that can clinically and radiographically mimic breast carcinoma. The most common clinical presentation is an unilateral, discrete breast mass, nipple retraction and even a sinus formation often associated with an inflammation of the overlying skin. The etiology of idiopathic granulomatous mastitis is still obscure. Its treatment remains controversial. The cause may be the autoimmune process, infection, a chemical reaction associated with oral contraceptive pills, or even lactation. Various factors, including hormonal imbalance, autoimmunity, unknown microbiological agents, smoking and α 1-antitrypsin deficiency have been suggested to play a role in disease aetiology. In this review, causing factors in the aetiology of idiopathic granulomatous mastitis are reviewed in detail.

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Key words: Mastitis; Granulomatous mastitis; Idiopathic granulomatous mastitis; Granulomatous lobular mastitis; Inflammation

Core tip: Aetiology of idiopathic granulomatous mastitis has not been fully elucidated. In this article, possible aetiological factors mentioned in the literature are discussed in detail. Additionally, ethnicity factor which is briefly mentioned previously in the literature are detailed.

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INTRODUCTION

Inflammatory events are frequently seen in the breast, and its can appear in a manner that clinically mimics malignancy, but they are usually benign. In addition, aetiological factors (trauma and breast-feeding) are generally identified after a detailed anamnesis. In cases where the aetiology is defined, it is easy to practice treatment algorithms starting with “limiting or removing the aetiological factor”. In cases where the aetiological factor is unknown, the diagnosis, differential diagnosis and treatment steps can become more complicated.

Since idiopathic granulomatous mastitis (IGM) was defined as a distinct clinical entity among benign breast diseases in 1972^[1], it has attracted clinicians’ attention, particularly those interested in breast diseases.

The differential diagnosis of IGM with breast cancer only clinically without histopathological examination is almost impossible. Therefore, presence of some complaints which can be seen in both disease (like palpable breast mass, nipple retraction, nipple skin hyperemia, erosion and fistula formation) would be more accurate to think of the first diagnosis of malignancy. In histopathological examination, presence of granulomatous inflammation and no malignancy is require performing other tests for the aetiology. Failure various factors which may result in granulomatous reaction in the breast (tuberculosis, certain

Table 1 Causes for granulomatous inflammatory reaction in the breasts

Infectious	Mycobacterium tuberculosis Blastomycosis Cryptococcosis Histoplasmosis Actinomycosis Filarial infection Corynebacterium
Autoimmune process	Wegener granulomatosis Giant cell arteritis Foreign body reaction
Duct ectasis	Plasma cell mastitis Subareolar granuloma Periductal mastitis
Diabetes mellitus	
Sarcoidosis	
Fat necrosis	
Idiopathic	

parasitic and fungal infections, Wegener's granulomatosis, giant-cell arthritis, polyarthritis nodosum, sarcoidosis, foreign body reaction, *etc.*) will support the diagnosis of IGM.

In this article, we discuss factors that may play a role in the aetiology of IGM.

DEFINITION

Idiopathic granulomatous mastitis (GM) is a rare, benign, chronic, inflammatory lesion of the breast, and its aetiology has not been fully elucidated. It was defined for the first time in 1972 by Kessler and Woollock and was described in detail in 1977 with a five-case series by Cohen^[1,2].

GM is generally divided into two main groups of specific and non-specific. The term "specific GM" refers to conditions for which the aetiological factor can be identified, whether an isolated inflammatory event only applies to the breast, or the breast is involved in a systemic inflammatory event. Nonspecific GM is also known as idiopathic granulomatous mastitis or granulomatous lobular mastitis, which generally refers to conditions that can lead to a granulomatous reaction in the breast or conditions for which the aetiological factors cannot be determined.

Chronic granulomatous inflammation constitutes 24% of all inflammatory events of the breast that are histopathologically defined^[3]. All factors that lead to granulomatous inflammation in the breasts are presented in Table 1^[4].

GENERAL REMINDERS

While IGM mostly emerges in young-middle age women (third and fourth decades), the age range that has been reported in the literature (11-83 years) is considerably wider^[5-7]. IGM is usually seen within a couple of years after giving birth, and the majority of patients have a history of at least one live birth and breast feeding^[8]. In con-

trast, specific GM is frequently seen in Asian and African countries and can be detected at any age^[9].

IGM may present with clinical findings that mimic the two endpoints of breast diseases such as breast abscess and breast cancer^[8]. A palpable mass in the breast is the most common complaint, but nipple retraction, hyperaemia in breast skin, oedema, ulceration and fistule development during the chronic period are also potential complaints^[9]. Systemic symptoms such as fever are generally not present^[6]. While the incidence is the same in both breasts, the lesion is usually unilateral and cases with bilateral involvement have been reported only rarely^[8,10,11].

PATHOGENESIS

The pathogenesis of IGM is not exactly known, but different steps occur in the disease pathogenesis. One of these steps is nonspecific lobulitis, which involves multiple lobules, and causes reactive lymphoplasmocytic infiltration. A granulomatous formation with central suppurative necrosis occasionally occurs because of lobule deformation. Abscesses develop because of an increase in the number of these foci^[12].

Some studies have indicated the similarity between IGM and granulomatous inflammation of the testicles or the thyroid gland when IGM was defined for the first time^[1]. Considering that mechanical factors are responsible for the formation of granulomas of the thyroid gland in multifocal granulomatous thyroiditis (palpation thyroiditis), the possibility that trauma represents another stage in IGM pathogenesis should not be disregarded^[12].

A process starting with non-puerperal secretion has been proposed as the most rational theory for the pathogenesis of IGM. A hormonal imbalance due to a deviation in the oestrogen-progesterone ratio or hyperprolactinemia is believed to cause this secretion and inflammation. Ductal ectasia occurs due to the intra-ductal accumulation of a protein-rich secretion. Permanent inflammation occurs following perforation of the ducti and contact between the secretion and stromal cells. The accumulation of secretion, ductal ectasia, galactoporitis (intraductal inflammation) and chronic GM are steps in the pathophysiological process. Autoimmunity against a secretion that is extravasated from the lobules is also considered to cause this event^[6,13,14].

Aetiology

The aetiology of IGM remains unclear. Various factors, including hormonal imbalance, autoimmunity, unknown microbiological agents, smoking and α 1-antitrypsin deficiency have been suggested to play a role in disease aetiology.

α 1-antitrypsin deficiency

α 1-antitrypsin (AAT) is a glycoprotein synthesised by hepatic cells. Similar to anti-thrombin 3, ovalalbumin and thyroid-binding globulin, AAT is a member of the serine-protease inhibitor family. Its primary function is to

prevent the destructive effects of proteases secreted from activated neutrophils (proteinase 3, elastin and cathepsin G). Because AAT level is elevated during inflammation, it is also accepted as an acute phase reactant. Deficiency in AAT leads primarily to lung and liver pathologies^[15]. In their case presentation in 2001, Schelfout *et al*^[16] demonstrated AAT deficiency in a 37-year-old female patient diagnosed with IGM. According to that study, the authors did not determine any other aetiological factors, and suggested that AAT deficiency could be the aetiological factor; however, further studies were not performed.

Oral contraceptives

The secretion theory has an important place in the pathophysiology of IGM. Oral contraceptives (OCS) have been considered a potential aetiological factor, as they increase breast secretion^[12]. However, a significant association between OCS and IGM has not been determined. Oran *et al*^[17] found 10 cases (10/46; 21.7%) that had a history of OCS use; Gurleyik *et al*^[18] found eight cases (8/19, 42.1%) that had a history of OCS use; and Al-Khaffaf *et al*^[19] found five cases (5/18, 27.7%) that had a history of OCS use. In contrast, Baslaim *et al*^[20] reported that none of 20 patients had a history of OCS use. Bani-Hani *et al*^[7] found that only two of 24 cases (8.3%) had a history of OCS use, and Asoglu *et al*^[21] found that only two of 18 cases (11.1%) had a history of OCS use. In conclusion, the association between IGM and OCS use has been reported to range between 0%-42%.

Gestation, birth, and breast-feeding

Given that IGM is usually detected in women < 50 years of age, and frequently involves a recent history of birth or breast-feeding, these factors have been considered in the disease aetiology. Hormonal alterations during these processes, secretion, and inflammation have an effect on disease pathophysiology^[19,21-28]. Bani-Hani *et al*^[7] carried out a study on 24 cases, and found that four had active gestation, four had a history of birth and breast-feeding within 6 months and only two cases did not have a history of gestation. According to a study by Baslaim *et al*^[20], all cases had a history of gestation and breast-feeding, whereas two cases were actively breast-feeding, and one case had an active gestation. Similarly, Gurleyik *et al*^[18] determined that four of 19 cases had a history of active breast-feeding, and the remaining 15 cases had a history of breast-feeding. Moreover, Oran *et al*^[17] reported that only three of 46 cases were nulliparous. Gautier *et al*^[14] conducted a case series study on 11 cases and emphasised that all cases except one male case had a history of birth and lactation within the past 5 years.

While almost all studies reported a history of parity, various studies have failed to explain the timing of the parity. It is expected that cases with IGM, which is a reproductive age disorder, have a history of gestation and breast feeding, as gestation occurs between the ages of 20-40 years. In addition to the male case, cases with a wide age range (11-83 years) in the literature make it

difficult to hold only gestation, birth and breast feeding responsible for the aetiology of IGM^[4,5,14].

Hyperprolactinemia

Considering the secretion theory, hyperprolactinemia has also been considered responsible for the pathogenesis of IGM, similar to other hormonal disorders^[12,29,30]. In a case presentation in 1984, Rowe^[29] determined co-morbid prolactinoma in an IGM case. However, future studies did not provide prolactin levels in detail. Bani-Hani *et al*^[7] analysed prolactin levels in seven of 24 cases and found elevated prolactin levels in one patient (4.1%). Erhan *et al*^[10] carried out a case-series study on 18 women and reported recurrence in three cases (16%), and identified hyperprolactinemia in two of these patients.

Smoking

While smoking is among the factors considered in the disease aetiology, a definitive association between smoking and IGM has not yet been established. According to a study by Asoglu *et al*^[21], 14 of 18 cases (77.8%) had a history of smoking, whereas Baslaim *et al*^[20] reported that none of their 20 cases had a history of smoking. In addition, the smoking rate was 34.8% according to Oran *et al*^[17], 16.7% according to Al-Khaffaf *et al*^[19], and 50% according to Ozel *et al*^[31].

Autoimmunity

A hypothesis that suggests an immunological basis for IGM has received considerable attention. Literature findings, including a good response to steroid and immunosuppressive treatment, patients who had recurrence after surgery showing a good response to steroid treatment, patients with extramammary involvement (such as erythema nodosum, or arthritis) and the demonstration of T-lymphocyte dominance in immunohistochemical studies support the autoimmunity hypothesis^[1,2,11-14,32]. Ozel *et al*^[31] conducted a study on eight cases and found that six were positive for rheumatoid factor (RF), and two were positive for anti-nuclear antibody (ANA) and anti-double stranded DNA (anti-dsDNA). In that study, surgery was the preferred treatment option for all patients, and the authors reported recurrence in two patients who were RF-, ANA- and anti-dsDNA-positive, but obtained a positive response after steroid treatment. Erhan *et al*^[10] conducted an immunohistochemical evaluation, and determined that 14 of 18 cases had T-cell dominance, and this finding was interpreted as an autoimmune pathophysiological outcome that progressed with reactive T-cell-mediated inflammation and centrilobular granulomas against ductal damage. Furthermore, two IGM cases with erythema nodosum, one IGM case with erythema nodosum and arthritis, one IGM case with Weber-Christian disease and one IGM case with Sjögren's syndrome have been reported in the literature^[33-36]. However, cases with a co-morbid autoimmune disorder constitute only a minor fraction of all cases. In contrast to these studies that support the autoimmune hypothesis, classical serological tests, which are used for au-

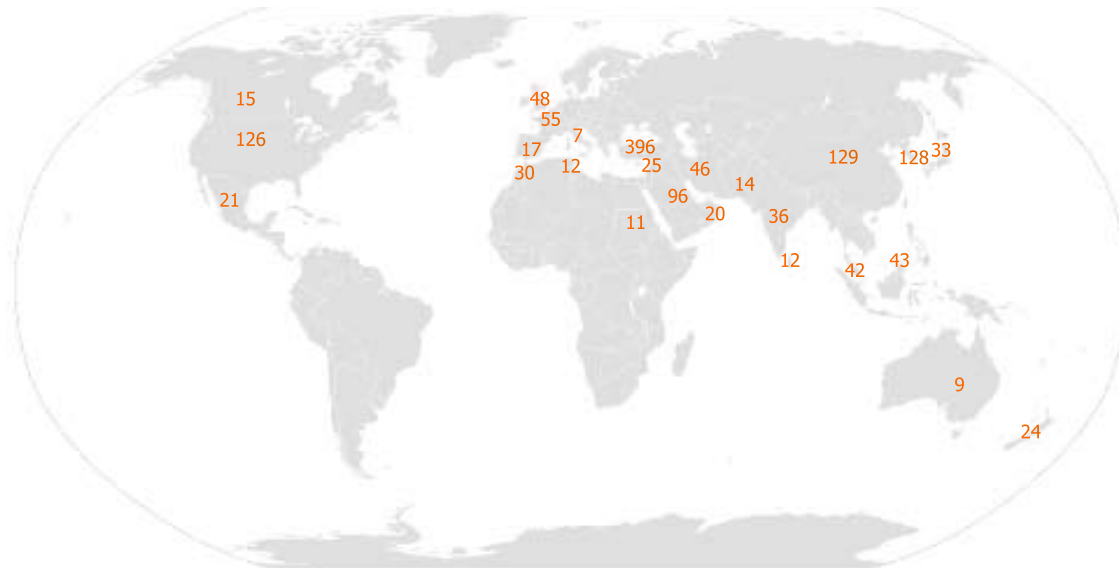


Figure 1 The distribution of idiopathic granulomatous mastitis cases that were reported in PubMed since 1995 according to country.

to immune disorders such as ANA and RF, reveal different results in patients with IGM. Asoglu *et al.*^[21] conducted a case-series study on 18 cases and determined that all cases were negative for ANA and RF. We conducted a study in our clinic to investigate the autoimmunity hypothesis for IGM aetiology, and evaluated ANA and extractable nuclear antibody levels in 26 cases, but we did not obtain results to support the autoimmunity hypothesis^[37].

Microbiological agents

The normal endogenous bacteria flora of the breast is similar to the skin flora. Dominant organisms include coagulase-negative streptococci, *Propionibacterium sp.* and *Corynebacterium sp.* These findings have been proven through nipple discharge and breast tissue cultures that were collected during mammoplasty^[38]. These bacteria are considered to go deeper into the breast tissue *via* the ductal system^[13].

Corynebacteria cause mastitis in livestock. However, these bacteria are not expected pathogens in humans^[13]. These bacteria became the centre of attention in 2003, with detection of corynebacteria in 34 IGM cases by Taylor *et al.*^[39].

Corynebacteria are Gram-positive bacteria and members of the skin flora. It is hard to distinguish whether these organisms cause infection, colonisation or contamination^[40]. Despite the difficulty in distinguishing outcome, it is significant to detect purulent matter in an abscess or > 10⁴ CFU/mL dominant *Corynebacterium sp.*^[41]. According to a study by Funke *et al.*^[42], these bacteria could be a possible factor if: (1) a Gram-positive bacillus accompanying polymorphonuclear leukocytes is present; or (2) a *Corynebacterium sp.* is detected in a tissue that is expected to be sterile under normal conditions.

Four different *Corynebacterium* species have been detected in IGM cases. *Corynebacterium kroppenstedtii* (*C. kroppenstedtii*) is the most frequently observed species, and

is different from other corynebacteria due to its lipophilic nature and positive esculin test^[39,40].

Taylor *et al.*^[39] conducted a study of 62 patients who were histologically diagnosed with GM, and detected *Corynebacterium* in 34 patients (54.8%). A comparison among the remaining 28 cases showed that fever and neutrophilia were more frequently observed in cases that were bacteria-positive, and they had more frequent fistule formation. *C. kroppenstedtii* was the most frequently observed species (14 patients; 41.1%) in that study.

Paviour *et al.*^[40] isolated *Corynebacterium* from breast tissue in 24 cases, carried out a histopathological evaluation in 12 of these cases and diagnosed nine cases with IGM. Similarly, *C. kroppenstedtii* was the most frequently isolated species in that study; *C. amycolatum* and *C. tuberculoostearicum* were other identified species. In that study, a 3-week intravenous penicillin treatment was tested on one patient; however, when the expected benefit was not observed, the treatment was switched to doxycycline (100 mg, oral), which has better fat solubility. The authors reported that there was no need for surgery after this treatment.

Case presentations in which *Corynebacterium sp.* have been detected are also present in the literature^[41,43,44]. A specific species was not reported in two of these studies, whereas Ang *et al.*^[41] reported that they isolated *C. accolens*. All three studies stated that antibiotherapy was effective for treatment.

In our clinic, we carried out a study on 45 patients with IGM and 34 bacteria using a universal DNA primer, but we did not detect positivity for any microbiological agent (unpublished data).

Ethnicity

During our search of GM in the PubMed database (1995-2014), we searched the terms “idiopathic granulomatous mastitis”, “granulomatous lobular mastitis” and

Table 2 The distribution of idiopathic granulomatous mastitis cases that were reported in PubMed since 1995 according to country

> 100 cases	20-100 cases	5-20 cases	< 5 cases
Turkey: > 200	Saudi Arabia: 96	Spain:17	Netherlands: 4
China: 129	France: 55	Canada: 15	Israel: 4
South Korea: 128	United Kingdom: 48	Pakistan: 14	Austria: 3
United States: 126	Iran: 46	Sri Lanka: 12	Belgium: 3
	Brunei: 43	Tunis: 12	Taiwan: 2
	Malaysia: 42	Sudan: 11	Caribbean: 2
	India: 36	Australia: 9	Peru: 1
	Japan: 33	Italy: 7	Nigeria: 1
	Morocco:30		Kuwait: 1
	Jordan: 25		Jamaica: 1
	New Zealand: 24		Greece: 1
	Mexico: 21		Norway: 1
	Oman: 20		

“granulomatous mastitis” and found approximately 200 articles. We hypothesised that an evaluation based on the location of the centres in which the authors worked would provide a rough estimate of the distribution of the cases. While most of these studies were case presentations, we found that larger case series frequently originated from the Mediterranean region and the developing countries in Asia. Some authors have considered that undiagnosed tuberculosis cases might lead to GM^[15,45-48].

According to our search on the PubMed database, the highest number of cases has been reported in Turkey (> 200 cases). This is followed by China (129 cases) and South Korea (128 cases). France had the highest number of cases (55 cases) among European countries, and no other country exceeded 50 cases. In contrast, we found 126 cases in the United States. The total number of cases recorded per country is presented in Table 2 and Figure 1.

According to a Centers for Disease Control and Prevention report, which was published in Morbidity and Mortality Weekly Report in 2009, seven cases were detected in Indiana between 2006 and 2008, and six of these cases were born in Mexico and had a Hispanic. According to the report, this series was the most comprehensive case series reported in the United States^[49]. However, Larsen *et al.* published a study on 54 cases in the same year, but the authors did not evaluate ethnicity^[50]. Gautier *et al.*^[15] carried out a study on 11 cases in Canada and reported that three cases were French, two cases were Canadian of French origin, two cases were Canadian of British origin, two cases were Latin American and one case was Russian. Furthermore, Omranipour *et al.*^[50] reported a series of 43 cases in Iran, Bani-Hani *et al.*^[7] reported a series of 24 cases in Jordan and Baslaim *et al.*^[20] reported a series of 20 cases in Saudi Arabia. In Turkey, different IGM series have been reported by Asoglu *et al.*^[21] (18 cases), Ozel *et al.*^[31] (8 cases), Gurleyik *et al.*^[18], Oran *et al.*^[17] (46 cases) and Altintoprak *et al.*^[37] (26 cases). These findings indicate that a previous comprehensive evaluation of ethnicity does not exist, and that more elaborate studies on this topic are required.

CONCLUSION

In conclusion, while several factors have been considered as potential aetiological factors, these factors are not ‘the primary aetiological factors, but rather “secondary factors” that can accompany the process once the primary factor triggers the event, or contribute to the acceleration’ of the ongoing process. Given that: (1) a higher number of cases are being reported from certain geographical locations; and (2) patients respond positively to steroid treatment, we believe that the “ethnicity and autoimmunity hypotheses” are the major subjects to focus on. It is possible that our failure in searching for a single aetiological factor will become more evident as details are elucidated; however, the disease is likely to continue to carry the “idiopathic” prefix for a long time.

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Role of immunotherapy in the treatment of allergic asthma

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Abstract

Allergen-specific immunotherapy (SIT) induces clinical and immunological tolerance as defined by persistence of clinical benefit and associated long-term immunological parameters after cessation of treatment. Although the efficacy of SIT has been shown in terms of reducing symptoms, medication consumption and ameliorating quality of life in both allergic rhinitis and asthma, there has long been some controversies about effectiveness of SIT in the treatment of allergic asthma. The type of allergen, the dose and protocol of immunotherapy, patient selection criteria, the severity and control of asthma, all are significant contributors to the power of efficacy in allergic asthma. The initiation of SIT in allergic asthma should be considered in case of coexisting of other allergic diseases such as allergic rhinitis, unacceptable adverse effects of medications, patient's preference to avoid long-term pharmacotherapy. Steroid sparing effect of SIT in allergic asthma is also an important benefit particularly in patients who have to use these drugs in high doses for a long-time. Symptomatic asthma is a risk factor for systemic reactions and asthma should be controlled at the time of administration of SIT. Both subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) have been found to

be effective in patients with allergic asthma. Although the safety profile of SLIT seems to be better than SCIT, the results of some studies and meta-analyses suggest that the efficacy of SCIT may appear better and earlier than SLIT in children with allergic asthma.

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Key words: Asthma; Efficacy; Safety; Subcutaneous immunotherapy; Sublingual immunotherapy

Core tip: Allergen specific immunotherapy is the only therapeutic approach that can change the immunologic response to allergens and thus can alter the natural evolution of allergic diseases. Both subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) have been demonstrated to be beneficial in reducing of symptoms and drug intake, improving quality of life and preventing patients from possible side effects of high doses of steroids. This review examines the clinical effectiveness and safety of both SCIT and SLIT in patients with asthma by discussing recent studies.

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INTRODUCTION

Asthma is one of the most prevalent chronic conditions affecting roughly 300 million people in the world. It is supposed that asthma will affect an additional 100 million people by 2025^[1].

According to data of health statistics in United States, current asthma prevalence is 9.3% and 8%, in children and adults, respectively^[2]. This increment in the prevalence of asthma has been accompanied by an increment in other allergic disorders like rhinitis and eczema.

Asthma is characterized by chronic inflammation,

which result in recurrent attacks of cough, wheezing, sometimes chest tightness and variable airflow obstruction. As time progresses, this airflow obstruction may become irreversible due to airway remodelling. Since many years, asthma has been supposed as mainly a Th2 cell-mediated disorder^[3,4]. Nevertheless, in recent years, it is also discovered that many other cell types such as Treg, Th1 and Th17 are also involved in pathological process of asthma^[3,4].

Drugs, such as inhaled corticosteroids, long-acting beta agonists and montelukast can effectively control asthma symptoms and attacks. However, it is known that, pharmacotherapy can not affect the underlying immune response; when these medications are stopped the symptoms may recur.

Specific allergen immunotherapy (SIT) is a unique therapy which capable to change the natural evolution of allergic diseases^[5]. With this treatment mode, allergens are given to patients in repeated and increasing doses to provide immune tolerance^[6].

The effectiveness of both subcutaneous (SCIT) and sublingual (SLIT) immunotherapy is documented for both perennial and seasonal allergic respiratory disease by systematic reviews and meta-analyses^[6-11]. For almost 100 years now, subcutaneous route has been used to treat allergic diseases; however, there are many studies to confirm the administration of SLIT because of discomfort of repeated injections and higher risk of adverse reactions.

In most published studies, effectiveness of SIT has been assessed primarily in patients with allergic rhinitis, and the results concerning asthma mostly were given as secondary outcome. Thus, there are a few studies which were organised to evaluate the efficacy of SIT specifically in asthma alone.

In this paper we will review primarily the clinical efficacy and safety of both SCIT and SLIT in patients with allergic asthma in the light of the literature.

CLINICAL EFFICACY OF SCIT IN ASTHMA

The first of the studies which evaluate the efficacy of SCIT in asthmatic patients published by Abramson in 1995^[12].

In the meta-analysis carried out by Ross *et al*^[13], 24 prospective, randomized, studies involving 962 asthmatic patients were evaluated. They reported significant amelioration in symptoms and drug intake related with asthma as well as in pulmonary function in the SCIT group in comparison to the placebo. It was deduced that immunotherapy was beneficial in 17 (71%) studies, inefficacious in 4 (17%) studies, and equivocal in 3 (12%) studies. Similar to the previous meta-analyses, the authors concluded that SIT is effective in patients suffering from allergic asthma.

In a study of Basomba *et al*^[14], 55 mild and moderate asthmatic patients (aged 14-50 years) allergic to house dust mites (HDM) were treated with *D pteronyssinus* ex-

tract encapsulated in liposomes, in a double-blind placebo-controlled manner. At the end of one year, 45.8% of the patients treated with SCIT decreased symptom and medication scores by at the minimum 60%. There were also notable improvements in results of skin test and allergen-specific bronchial challenge.

In another study, fifteen children aged 6-14 years with asthma due to HDM were treated with SCIT for three years; the results were remarkable reduction in the number of asthma exacerbations and marked decrease in drug intake^[15]. Additionally, significant improvement in lung functions and non-specific bronchial hyperreactivity (BHR) were observed.

García-Robaina *et al*^[16] administered SIT with HDM in 64 adult asthmatic patients and they observed notable amelioration in the active group over placebo in terms of symptom (53.8 %) and medication scores (58%) in addition to improvement in allergen-specific BHR.

Roberts *et al*^[17] studied the efficacy of grass pollen SIT in 35 asthmatic patients (aged 3-16 years) over 2 pollen season in a double-blind manner. They found that SIT provided significant decreases in asthma symptom and medication scores, marked improvements in cutaneous ($P = 0.002$), conjunctival ($P = 0.02$), and bronchial ($P = 0.01$) reactivity to allergen.

In the study of Zielen *et al*^[18], 65 mite allergic children aged 6-17 years were treated with subcutaneous allergoid immunotherapy plus fluticasone propionate (FP) or FP therapy alone for 2 years. Before starting SIT, asthma control was achieved using inhaled corticosteroids for 5 mo follow-up. Children treated with SCIT plus FP were able to markedly decrease the FP dose, in comparison to the control group given only FP. After 2 years of treatment, the mean daily FP dose decreased from 330.3 µg to 151.5 µg in the immunotherapy group while there was no significant reduction in the control group.

In a recent Cochrane review of SCIT, 88 studies on 3459 subjects with asthma were evaluated; there were 42 trials for dust mites, 27 for pollen, 10 for animal dander, two for molds, two for latex, and six for multiple allergens^[19]. It was reported that SCIT improved asthma symptoms, reduced medication use, and diminished BHR. The conclusion of this review was summarized as: "it would require treating three subjects to prevent an exacerbation for one individual, four subjects to improve medication use in one, and four subjects to avoid nonspecific or allergen-specific BHR in one patient, respectively". Additionally, mite and pollen immunotherapy were found more effective on symptom scores.

There are several studies of SCIT (particularly with mites^[20,21] or mixed-allergen up to seven aeroallergens^[22]) which demonstrated the improvement in asthma symptom and medication scores to a lesser degree than the other published studies. Nevertheless, significant steroid-sparing effect of immunotherapy was shown in moderate persistent asthmatics included in those studies. Thus, it should be kept in mind that the maintenance of asthma control is very important before and during the study in order to obtain optimal benefit of the immunotherapy.

CLINICAL EFFICACY OF SLIT IN ASTHMA

World Allergy Organization Position Paper on Sublingual Immunotherapy declared that SLIT is effective in the treatment of allergic rhinitis in adults and in allergic rhinitis and asthma in children^[23]. However, it is also stated the presence of some important points about current status of SLIT effectiveness. It is known that there are significant heterogeneity between studies included in SLIT meta-analyses, and this may bring significant limitation on the conclusion of them.

The first meta-analysis on SLIT in asthma was conducted by Olaguibel *et al.*^[24] and comprised of seven studies in 256 children aged up to 14 years. This study showed marked improvements in symptom scores (SMD: -1.42) and medication requirement scores (SMD: -1.01) related with asthma.

In 2006, a meta-analysis about SLIT in asthma included 25 trials and involved 1706 adults and children^[25]. This meta-analysis reported a significant efficacy of SLIT for symptoms and medication use in seven studies, and improvement in pulmonary function in four studies. But, when asthma symptoms and drug intake were analysed as ongoing parameters, the reductions were not significant.

Penagos *et al.*^[26] evaluated the efficacy of SLIT by conducting a meta-analysis which included nine studies on 441 asthmatic children. Six of these studies were with mites and three of them with pollen. The authors found significant decrease in symptom and medication scores with SLIT in comparison to placebo.

In 2009, Compalati *et al.*^[10] published a meta-analysis which evaluate nine studies in 452 patients treated with SLIT in HDM-allergic asthma. They reported marked improvement in symptom and medication scores related with asthma. As in SCIT, the steroid sparing effect of SLIT was also demonstrated in some recent published studies^[27,28].

In the study of Marogna *et al.*^[28], 84 asthmatics were randomized to four treatment arms for three years: first group received budesonide 800 µg/d; second group received budesonide 1600 µg/d; third group treated with budesonide 400 µg/d plus montelukast 10 µg/d; and fourth group was given budesonide 400 µg/d plus allergoid of betulaceae pre-coseasonally. Low-dose inhaled corticosteroids plus SLIT provided a marked advantage over the other options on symptoms plus medications decrease, FEV1 increase, rescue medications usage, and was comparable to low-dose inhaled corticosteroids plus montelukast on MEF25 and BHR.

Similarly, in a study involving 602 mite allergic asthmatic patients, it was shown that daily treatment with SLIT tablet reduced inhaled budesonide more than 80 µg/d in comparison to placebo after 1 year^[27].

HEAD-TO HEAD STUDIES

There are 4 randomized controlled trials with 171 participants which compare SCIT with SLIT directly in asthmatic patients. All these studies enrolled mite allergic

patients with rhinitis and/or asthma. Efficacy of SIT was investigated by evaluating the clinical outcomes for both rhinitis and asthma.

In the first of these studies, Mungan *et al.*^[29], randomized 36 adults with HDM-allergic rhinitis and asthma to receive SCIT, SLIT or placebo. They found that one-year of SCIT improved symptom scores of both rhinitis and asthma while SLIT had benefit only on symptoms of rhinitis. However, medication scores of both rhinitis and asthma decreased significantly in both actively treated groups. After 1 year of immunotherapy, it was also shown marked rises in specific IgG4 concentrations in comparison to the baseline both in SLIT and SCIT groups.

Eifan *et al.*^[30] evaluated the effectiveness of SCIT and SLIT in children with asthma/rhinitis sensitized to mites. Forty eight children were randomized to treat either SCIT, SLIT or pharmacotherapy. This study demonstrated that both SLIT and SCIT have a significant positive effect on symptoms and medication usage related with both rhinitis and asthma in comparison to the pharmacotherapy group. Additionally, after 1 year of treatment, Der p 1-driven IL-10 significantly increased in SLIT in comparison to pharmacotherapy, whereas Bet v 1-driven TGF-β increased significantly in SLIT only.

In the study of Keles *et al.*^[31], 48 patients (aged 5-10 years) with mild persistent asthma and rhinitis mono-sensitized to mites were randomized to three treatment arms: they received either SLIT ($n = 16$), SCIT ($n = 16$) or pharmacotherapy alone ($n = 16$). After 12-mo of treatment, total asthma symptom scores ($P = 0.02$) and visual analog scores ($P = 0.02$) decreased markedly in SLIT when compared with the pharmacotherapy group. Similarly, SCIT also reduced both total asthma symptom-scores ($P = 0.04$) and visual analog scores ($P = 0.001$) when compared with the pharmacotherapy group. The percentage of improvement was 100% and 93% in SLIT and SCIT group respectively, in comparison to the pharmacotherapy group. A marked increment was seen in the levels of regulatory and Th1 cytokines both in the SCIT and SLIT groups. Antigen-specific IgG4 levels increased in the SCIT and SCIT plus SLIT groups but not in the SLIT group.

In a recent randomized, placebo-controlled and double-dummy study we investigated the effectiveness of SCIT and SLIT in HDM-allergic children with asthma and/or rhinitis^[32]. We showed that one-year SCIT had significant effect on symptom and medication scores related with both rhinitis and asthma. An important observation in this study was the better effect of only SCIT over placebo on reduction of rhinitis and asthma symptoms at the end of one-year-treatment. Bronchial challenge doses and sputum eosinophil increments after bronchial challenge decreased only with SCIT. There was no change in terms of IFN-γ levels in both immunotherapy groups. Serum sIgG4 levels increased significantly only in the SCIT group. This study then carried on one subsequent year in an open scheme and the placebo group was randomized to treat SCIT or SLIT. Thus, all patients

received active treatment with SCIT or SLIT during one subsequent year^[33]. We observed that the effect of SLIT on asthma symptoms and drug intake was less eminent than SCIT in the first year; however this effect was more pronounced in the second year of SLIT. With this study, we concluded that both clinical and immunologic improvement starts earlier with SCIT in comparison to the SLIT in mite-allergic children with rhinitis and asthma.

The summary of these 4 head-to-head studies was shown in Table 1. Recently, a systematic review of studies with head-to-head comparison of SCIT and SLIT in the treatment of allergic rhinoconjunctivitis and asthma was published^[34]. Four trials conducted in patients with rhinitis and/or asthma^[29-32]. This review demonstrated that low-grade evidence confirms more efficacy of SCIT than SLIT regarding reduction of asthma symptoms and combined measure of rhinitis symptoms and drug intake; moderate-grade evidence confirms more efficacy of SCIT than SLIT for nasal and/or eye symptom reduction. It was deduced that low-grade evidence confirms that SCIT is more beneficial than SLIT for reduction in asthma symptoms and moderate-grade evidence for reduction of allergic rhinoconjunctivitis. Further studies are required to support this results for clinical decision making.

SAFETY OF SCIT AND SLIT

It is known that SCIT has a risk for both local and systemic adverse reactions but, in most of the cases, symptoms are reversible if they are diagnosed early and treated rapidly. All allergen preparations (standardized extracts^[35], allergoids^[36] or recombinant allergens^[37] can cause these side effects.

The incidence of systemic reactions of SCIT varies between 0.06% and 1.01% in those receiving injections^[38].

A recent multicenter study suggested that systemic reactions were slightly more frequent in rhinitis with asthma than rhinitis patients alone^[39]. Some reports have been suggested that asthma may be a risk factor for severe systemic reactions due to SCIT, notably in patients with uncontrolled asthma. Conversely, another retrospective study reported no significant association between systemic reactions and the presence of asthma^[40]. As noted by official documents, the patients's general condition and pulmonary functions should be assessed before injection in order to reduce the risk of anaphylaxis^[41].

The safety of SLIT seems better than subcutaneous therapy regarding severe systemic reactions. Local side effects (oral itching or mild swelling) may be encountered in three-fourths of patients especially in the early phase of SLIT.

In the study of Dahl *et al*^[42] the safety of SLIT investigated specifically in grass pollen allergic patients with asthma. They evaluated side effects which may be related with asthma, *e.g.*, cough, wheezing, and they found no difference in the number of such effects between active and placebo group. Additionally, no asthma exacerbation

related with SLIT was reported in this study.

There are also some recommendations about administering of SLIT in patients with systemic reactions after subcutaneous immunotherapy^[43]. Nonetheless, some patients suffering from these adverse reactions with subcutaneous route may entertain the same risk for sublingual route of immunotherapy^[44]. Thus, our recommendation is that immunotherapy should be customized to each patient on the basis of the degree of sensitization, concomitant allergies, exposures and patient's preference.

PREVENTIVE CAPACITY OF SIT

SIT builds up clinical and immunological tolerance as shown by persistence of improvement both in clinical and immunologic parameters after the cessation of treatment. Additional long-term benefits of SIT include prevention of new sensitizations and progression from rhinitis to asthma.

There are some studies which demonstrated the preventive effect of SIT in pediatric population. At the 10-year follow-up (7 years after cessation of immunotherapy) the children in the immunotherapy group had significantly less asthma in comparison to the control group: 16/64 (25%) with asthma in the immunotherapy group compared with 24/53 (45%) of the untreated control group^[45]. The authors concluded that immunotherapy for 3 years with grass and/or birch allergen extracts provides long-term preventive effect on the development of asthma in children with only seasonal rhinoconjunctivitis.

A similar preventive effect was also shown with SLIT in a 3-year open study of 113 children (aged 5-14 years) having grass pollen rhinitis^[46]. This study demonstrated that asthma development was 3.8 times more frequent in the control subjects.

There is another study which show no significant difference in symptom and medication scores in the subsequent three pollen seasons after 3-4 years of grass-pollen SCIT^[47].

Marogna *et al*^[48] have noted that clinical benefit persists for 8 years after SLIT treatment is given for a 4- to 5-year duration; new sensitizations were also reduced in SLIT group.

It has been documented that SCIT with a single allergen has a preventive effect against sensitization to different inhalant allergens^[49-52]. There are some studies which reported significantly lower rate of the development of new allergen sensitizations in monosensitized patients who received SCIT in comparison to the controls^[49-52]. In these studies, the percentage of the development of new sensitizations were 23%, 24%, 24.7% and 54% in patients treated with SCIT while 68%, 67%, 53.3% and 100% in untreated monosensitized patients.

Recent studies have shown such effects with SLIT^[48,53-55]. In a 3-year open study, 5.9 % of 511 patients with allergic rhinitis and asthma treated with SLIT showed new allergen sensitizations, while this rate was 38% in the control patients^[55].

Table 1 Head-to-head studies which included patients with asthma treated by subcutaneous and sublingual immunotherapy¹

Ref.	Year	Study Design	Age	No of patients	Asthma symptom score				Medication score				Findings
					Before SIT		After SIT		Before SIT		After SIT		
					SCIT	SLIT	SCIT	SLIT	LSCIT	SLIT	SCIT	SLIT	
Mungan <i>et al</i> ^[29]	1999	Single-blind, placebo controlled	Adults	SCIT (<i>n</i> = 10) SLIT (<i>n</i> =15) Placebo (<i>n</i> = 11)	1.2	0.63	0.59	0.41	6.8	4.93	3.9	1.97	Reduction in symptom scores with only SCIT Reduction in medication scores with both SCIT and SLIT
Eifan <i>et al</i> ^[30]	2010	Open label, randomized, controlled	5-10	SCIT (<i>n</i> = 16) SLIT (<i>n</i> = 16) Pharmacotherapy (<i>n</i> = 16)	0.9 ± 0.7	1.4 ± 1.5	0.4 ± 0.6	0.2 ± 0.4	2.4 ± 1.4	2.8 ± 1.2	1.7 ± 1.4	1.2 ± 0.9	Reduction in symptom and medication scores and visual analog scores with both SCIT and SLIT
Keles <i>et al</i> ^[31]	2011	Open label, randomized, controlled	5-12	SCIT (<i>n</i> = 11) SLIT (<i>n</i> = 13) SCIT plus SLIT (<i>n</i> = 14) Pharmacotherapy (<i>n</i> = 12)	0.25	0.12	0	0	0.52	0.69	0.06	0.23	Reduction in symptom scores and visual analog scores with both SCIT and SLIT
Yukselen <i>et al</i> ^[32]	2012	Randomized, double-blind, double-dummy, placebo-controlled	6-14	SCIT (<i>n</i> = 10) SLIT (<i>n</i> = 11) Placebo (<i>n</i> =10)	2.4	3.7	1	2.7	2.3	2.3	1	1.7	Only SCIT was found superior to placebo on reduction of symptom and medication scores

¹All studies used HDM immunotherapy. SCIT : Subcutaneous immunotherapy; SLIT: Sublingual immunotherapy; SIT: Specific immunotherapy.

CONCLUSION

SIT is the only therapeutic approach which capable to modify the natural evolution of allergic respiratory diseases. However, there are some shortcomings in trials conducted in patients with allergic asthma. In most of these studies, efficacy of SIT was not evaluated specifically in allergic asthma alone. Additionally, many of these trials had significant limitations such as low number of patients, difference in treatment protocols and doses, inadequate evaluation of pulmonary functions or absence of a placebo group. Moreover, there is a great heterogeneity between studies included in meta-analyses; the most important point in this respect is the assessment of results of SIT with different allergens in the same meta-analysis.

Despite these shortcomings, the clinical efficacy of SIT has been established in allergic asthma in objective and subjective parameters such as titrated skin tests, allergen-specific bronchial hyperreactivity, and symptom and medications scores.

Steroid sparing effect of SIT gives an important advantage for patients who have to use these drugs in high doses in order to control their asthma symptoms for many years.

SIT should be considered in asthmatic patients who experience side effects of medications, to reduce or avoid long-term pharmacotherapy and the economic burden of medications and in the presence of allergic rhinitis and/or other comorbid allergic conditions^[41].

Official documents recommend that SIT should not be started in patients with unstable asthma; in these cases, SIT can be initiated after well asthma control with appropriate pharmacotherapy.

Although both SCIT and SLIT have been reported to be effective on allergic asthma, the results of some studies or meta-analyses suggested that the efficacy of SCIT may be better and start earlier than SLIT.

Further studies are needed to discover patients who will benefit more from immunotherapy, novel vaccines and new routes of administration to increase efficacy and safety.

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Precancerous lesions of oral mucosa

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alternatives such as corticosteroids, calcineurin inhibitors, and retinoids are widely used.

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Key words: Oral premalignant lesions; Leukoplakia; Erythroplakia; Submucous fibrosis; Lichen planus; Malignant transformation

Core tip: Precancerous lesions of oral mucosa are the diseases that have malignant transformation risk at different ratios. Clinically, these diseases may sometimes resemble each other. Thus, the diagnosis should be confirmed by biopsy. In early stages, histopathological findings are distinctive, but if malignant transformation occurs, identical histological features with oral carcinoma are seen. If these diseases left untreated, they can cause many problems, which may affect a patient's social and daily life.

Abstract

Precancerous lesions of oral mucosa, known as potentially malignant disorders in recent years, are consists of a group of diseases, which should be diagnosed in the early stage. Oral leukoplakia, oral submucous fibrosis, and oral erythroplakia are the most common oral mucosal diseases that have a very high malignant transformation rate. Oral lichen planus is one of the potentially malignant disorders that may be seen in six different subtypes including papular, reticular, plaque-like, atrophic, erosive, and bullous type, clinically. Atrophic and erosive subtypes have the greater increased malignant transformation risk compared to another subtypes. Although there are various etiological studies, the etiology of almost all these diseases is not fully understood. Geographically, etiologic factors may vary. The most frequently reported possible factors are tobacco use, alcohol drinking, chewing of betel quid containing areca nut, and solar rays. Early diagnosis is very important and can be lifesaving, because in late stages, they may be progressed to severe dysplasia and even carcinoma *in situ* and/or squamous cell carcinoma. For most diseases, treatment results are not satisfactory in spite of miscellaneous therapies. While at the forefront of surgical intervention, topical and systemic treatment

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INTRODUCTION

In a World Health Organization Workshop, held in 2005, the terminology, definitions and classifications of oral lesions with a predisposition to malignant transformation have been discussed and recommended to use the term “potentially malignant disorders” to eliminate terminological confusion^[1].

The most common oral precancerous lesions are oral leukoplakia, oral submucous fibrosis (OSMF), and oral erythroplakia. Actinic cheilitis, some miscellaneous inherited diseases such as xeroderma pigmentosum and Fanconi's anemia, and immunodeficiency are another potentially malignant disorders for oral carcinoma as well as these three diseases^[1,2]. In a clinicopathological study



Figure 1 White and red lesions known as erythroleukoplakia are seen.

designed by Phookan *et al.*^[3], different oral premalignant lesions were observed in 70 of 320 patients with lower or middle socioeconomic group. Leukoplakia was the most common premalignant disorder (20.65% of patients), while percentages of lichen planus and OSMF were reported equally (0.62% of patients)^[3].

The etiology of precancerous lesions of oral mucosa is not well-known^[4]. Some risk factors such as tobacco chewing, tobacco smoking, and alcohol play an important role in development of potentially malignant oral conditions. While tobacco chewing is a major risk factor for oral leukoplakia, OSMF, and erythroplakia, tobacco smoking may be a risk factor for oral leukoplakia. Alcohol drinking may increase the risk by 1.5-fold for oral leukoplakia, by 2-fold for OSMF, and 3-fold for erythroplakia. According to Thomas *et al.*^[2], while alcohol drinking and tobacco chewing may possibly be risk factors for multiple oral premalignant lesions, smoking was not associated with the risk of multiple oral premalignant lesions.

Various studies reported about etiopathogenesis of precancerous lesions of oral mucosa. Vlková *et al.*^[4] analyzed saliva markers of oxidative stress and reported that salivary thiobarbituric acid reacting substances and advanced glycation endproducts were significantly higher in patients than in control. They also reported that no significant differences were found in salivary advanced oxidation protein products, vascular endothelial growth factor, sialotransferase, and neuraminidase. Total antioxidant capacity and expression of superoxide dismutase were lower in patients than in age-matched controls. Nanda *et al.*^[5] investigated expression of CK8 and CK18 in potentially malignant disorders such as oral leukoplakia, OSMF, and oral squamous cell carcinoma and they reported that expression of CK8 and CK18 were statistically significant higher than controls. Feng *et al.*^[6] reported that expression of podoplanin and ABCG2 in oral erythroplakia correlate with oral cancer development. Qin *et al.*^[7] reported a high prevalence of p53 mutations in premalignant oral erythroplakia. Human papilloma virus (HPV) has been suggested to play a role in the etiopathogenesis of precancerous lesions of oral mucosa^[8]. Punyani *et al.*^[9] reported that there was no statistically significant in salivary IL-8 concentrations among the precancer group and controls.

Table 1 Risk factors of malignant transformation

Female gender
Long duration of leukoplakia
Leukoplakia in non-smokers
Location on the tongue and/or floor of the mouth
Size > 200 mm ²
Non-homogenous type
Presence of epithelial dysplasia

Early detection of premalignant lesions and oral cancer is very important. Therefore, miscellaneous modalities such as oral cavity examination, supravital staining, oral cytology and optical technologies including spectroscopy, fluorescence spectroscopy, elastic scattering (reflectance) spectroscopy, Raman spectroscopy, fluorescence imaging, optical coherence tomography, narrow-band imaging, and multimodal optical imaging may be used^[10].

We think that the following criteria should be taken into consideration in terms of the importance of early diagnosis: (1) symptomatic and/or non-symptomatic non-healing lesions of oral mucosa; (2) history of smoking, chewing tobacco, alcohol consumption, oral HPV infection, drug use, long-term exposure to sunlight; (3) advanced age; (4) the presence of immunodeficiency; (5) the presence of genetic disease; and (6) poor oral hygiene.

ORAL LEUKOPLAKIA

Leukoplaki is defined as “A white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer”^[1]. In studies reported in recent years, the prevalence of oral leukoplakia varies between 1.1% and 11.7%, with a mean value of 2.9%^[11]. Although leukoplakia can occur at any age, it often occurs in individuals under the age of 40^[12]. Leukoplakia is seen six times more among smokers than among non-smokers^[1].

Clinically, leukoplakia may be affect any part of the oral and oropharyngeal cavity and can be divided into two subtypes including homogeneous and non-homogeneous types^[1]. Homogenous lesions are characterized by uniformly flat, thin, uniformly white in colour and shows shallow cracks of the surface keratin^[1,13]. Non-homogenous lesions have been defined as a white and red lesion (known as *erythroleukoplakia*) that may be either irregularly flat (speckled) or nodular (Figure 1). Verrucous leukoplakia is yet another type of non-homogenous leukoplakia^[14].

Proliferative verrucous leukoplakia, which is a form of verrucous leukoplakia, was first described by Hansen *et al.*^[14,15] in 1985 and characterized by multifocal presentation. It has a strong potential for malignant transformation and is resistance to treatment.

Histopathologically, two distinct appearances may be seen as dysplastic or non-dysplastic leukoplakia. Risk factors of malignant transformation are shown in Table 1^[14].

Oral leukoplakia should be distinguished from miscellaneous benign and/or potentially malignant disorders

that may be seen white or predominantly white diseases of the oral mucosa. The diseases should be considered in the differential diagnosis including aspirin burn, chemical injury, oral pseudomembranous and hyperplastic candidiasis, frictional lesions, oral hairy leukoplakia, leukoedema, linea alba, lupus erythematosus, morsicatio buccarum, papilloma and allied lesions, mucous patches in secondary syphilis, tobacco-induced lesions, smoker's palate (nicotinic stomatitis), stuff-induced lesion, white sponge nevus, oral lichen planus (OLP), and lichenoid reaction^[1,13].

Oral leukoplakia should be confirmed by mucosal biopsy. But before biopsy, some staining methods may be used as a diagnostic aid. Chen *et al*^[16] used methylene blue in fifty-eight patients with suspicious oral cavity lesions. They reported that the overall sensitivity of methylene blue uptake in cases with suspected lesions was 90%, specificity 69%, and accuracy 79%. They also reported that the positive predictive value was 74% and the negative predictive value 87%^[16].

The most commonly preferred treatment options are surgical excision or CO₂ laser therapy. In widespread lesions, photodynamic therapy may be considered^[14]. Cryotherapy is another preferred destructive method^[17]. Non-surgical treatment modalities might be considered in selected patients. Carotenoids (β -carotene, lycopene), vitamins [L-ascorbic acid (vitamin C), α -tocopherol (vitamin E), retinoic acid (vitamin A), and fenretinide], and bleomycin may be used in patients with oral leukoplakia^[18].

Surgical excision should be recommended in the presence of moderate and severe epithelial dysplasia. Reported recurrence ratios after surgery treatment have been varied between 10% and 35%^[18]. Kawczyk-Krupka *et al*^[19] compared to efficacy of cryotherapy and photodynamic treatment and reported that complete responses were obtained in 72.9% and 89.2% of patients in groups treated by photodynamic treatment and cryotherapy, respectively. Recurrence ratios were reported as 27.1% and 24.3% in groups treated by photodynamic treatment and cryotherapy, respectively^[19]. Pietruska *et al*^[20] reported significant reduction (on average by 53.8%) of leukoplakia lesions sizes after photodynamic therapy. Among patients treated by topical retinoic acid, while complete response ratio was reported between 10% and 27% of patients, partial response ratio was reported between 54% and 90% of patients. Recurrence of leukoplakia was reported as approximately 50% after withdrawing the topical retinoic acid^[21].

ORAL ERYTHROPLAKIA

Erythroplakia is defined as "A fiery red patch that cannot be characterized clinically or pathologically as any other definable disease". Clinical appearance is characterized by flat or even depressed erythematous change of the mucosa without a patch lesion. Both red and white changes in the same lesion refer to as "*erythroleukoplakia*". Prevalence of erythroplakia varies between 0.02% and 0.83%. It mainly occurs in the middle aged and the elderly. Male gender is most frequently affected. Mostly, a solitary le-

sion occurs over the surface of any part of the oral cavity. But the most commonly affected areas were reported as the soft palate, the floor of the mouth, and the buccal mucosa^[14,22].

Etiopathogenesis is not known exactly^[22]. Chewing tobacco and alcohol use are the possible etiologic factors for the development erythroplakia. Hashibe *et al*^[23] reported that chewing tobacco and alcohol drinking are strong risk factors for erythroplakia in the Indian population. High prevalence of p53 mutations in premalignant oral erythroplakia was reported in a study designed by Qin *et al*^[7].

Clinically, typical lesion of oral erythroplakia is less than 1.5 cm in diameter, but it also be less than 1 cm and larger than 4 cm^[22]. Histopathologically, moderate or severe dysplasia was usually seen in lesion with erythroplakia. Malignant transformation rates is very high (vary from 14% to 50%), so it needs to be treated expeditiously^[14,22].

Oral erythroplakia should be diminished from any disease which clinically appears red colour in oral cavity. Oral candidiasis, oral histoplasmosis, oral tuberculosis, atrophic OLP, lupus erythematosus, pemphigus, pemphigoids, amelanotic melanoma, haemangioma, telangiectasia, lingual varices, Kaposi's sarcoma, early squamous cell carcinoma, local irritation, mucositis, drug reaction, median rhomboid glossitis, and oral purpura may be confused with oral erythroplakia^[22,24].

Owing to the high malignant transformation rate, early effective treatment is mandatory^[22]. Surgery, either by cold knife or by laser, is the recommended therapy^[1]. Surgical excision may be used in lesions with severe epithelial dysplasia or carcinoma *in situ*^[22].

OLP

Lichen planus was first described by Erasmus Wilson in 1869^[25]. The disease is a chronic, autoimmune, inflammatory disease which may affect skin, oral mucosa, genital mucosa, scalp, and nails^[26]. Prevalence of OLP varies from 0.5% to 3%^[25]. It mainly occurs among female gender and the age of onset is usually between third and sixth decade^[25,27].

Although it is believed that OLP is a T-cell mediated autoimmune disease, its cause is partially understood in most cases^[28]. Several factors have been proposed for the etiology including genetic background, dental materials (amalgam, metals, gold, and composite restorations), drugs (especially antimalarials, cardiovascular agents, gold salts, non-steroidal anti-inflammatory drugs, hypoglysemics), infectious agents (herpes simplex virus, Epstein-Barr virus, cytomegalovirus, herpes virus-6, hepatitis-C virus, and human papilloma virus), autoimmunity, immunodeficiency, food allergies, stress, habits, trauma, diabetes and hypertension, malignant neoplasms, and bowel disease^[26,29,30].

Even though OLP may affect any part of the oral mucosa, most commonly affected areas are dorsum of the tongue (Figure 2), buccal mucosa (Figure 3), and gin-



Figure 2 Liken planus lesions on the dorsum of the tongue.



Figure 3 Reticular pattern lesions on the buccal mucosa.

giva^[26]. Clinically, OLP may be seen as six types including papular, reticular, plaque-like, atrophic, erosive, and bullous type^[25]. The most common type is the reticular pattern which present as fine white striae known as “*Wickham's striae*”. Typically, lesions present symmetrically and bilaterally, and usually asymptomatic. Atrophic pattern presents as a red lesion. Erosive pattern is usually seen as irregular erosion or ulceration covered with a fibrinous plaque or pseudomembrane. Both atrophic and erosive pattern are generally associated with a burning sensation and pain that exacerbated by trauma and hot, spicy or acidic foods. Plaque type clinically resembles leukoplakia because of its homogenous white nature. The dorsum of the tongue and buccal mucosa are the most affected areas in the oral cavity of patients with plaque type OLP. Multifocal plaque type lesions may be seen. This subtype is more common among tobacco smokers. The papular pattern, which is rarely seen, is characterized by small, white, raised papules with fine white striation at the periphery of the lesion. Bullous pattern is the least common type of OLP that characterized by bullae formation range from a few millimeters to several centimeters in diameter^[26].

In 1906, Dubrell first described the histologic features of OLP, but within the next years, it has been revised. Diagnostic histologic features include liquefactive degeneration of the basal cells, colloid bodies (known as *Civatte* bodies), homogenous infiltrate of lymphocytes in a dense, band-like pattern along the epithelium-connective tissue interface in the superficial dermis, cytologically normal maturation of the epithelium, sawtooth rete ridges, and hyperkeratosis. In erosive lichen planus, ulceration may be seen in the surface epithelium^[31].

The first case of OLP-related oral carcinoma was reported by François Henri Hallopeau in 1910. Malignant transformation ratio has been reported in 0% to 10% of patients, according to the sample's characteristics and study design, after mean follow-up of 1.5 to 10 years^[25]. Increased malignant transformation risk occurs greater in erosive and atrophic forms and in cases of lesions of lateral border of the tongue^[27].

If there are Wickham's striae typically, the diagnosis is easy and can be made clinically, especially reticular

pattern of OLP. But erosive or atrophic pattern need to be confirmed by biopsy in order to make the correct diagnosis^[32]. Direct immunofluorescence may be useful to distinguish from some bullous diseases such as pemphigus vulgaris, benign mucous membrane pemphigoid, and linear immunoglobulin A (IgA) bullous dermatitis^[31]. IgA, IgG, IgM or C3 deposition throughout the basement membrane and irregular fibrinogen deposition in the basement membrane are the diagnostic immunofluorescence findings in OLP and positivity rate is 65.8% of the patients with OLP^[33]. Indirect immunofluorescence studies are not useful in terms of diagnosis^[32].

Patients with reticular and other asymptomatic OLP can be followed without treatment. But if there are any symptoms and/or potential malignant risk, lesions should be treated. Both topical and systemic treatment modalities have been reported for OLP, shown in Table 2^[31,34-36].

ORAL SUBMUCOUS FIBROSIS

Oral submucous fibrosis, was first described by Schwartz in 1952, is chronic and potentially malignant disorder characterized by juxtaepithelial fibrosis of the oral cavity. Fibroelastic change of the lamina propria and epithelial atrophy occur in consequence of juxta epithelial inflammatory reaction, and eventually, stiffness of oral mucosa, trismus and an inability to eat develops^[37].

OSMF is usually seen in Asians population (particularly Indians) from the southern states and Taiwanese. Predominantly, it occurs in the second and third decade, and both sexes may be affected^[37]. But in patients with pediatric age group were rarely seen^[38-40]. Paymaster firstly described its premalignant nature in 1956. This malignant transformation rate was reported 7%-30%^[37].

Its etiology is not well-known and thought to be multifactorial^[37]. The strongest risk factor for OSMF is the chewing of betel quid containing areca nut. Other factors like genetic and immunologic predisposition also play a role in OSMF because of reported in families whose members are not in the habit of chewing betel quid or areca nut^[41]. Ranganathan *et al.*^[42] designed a case-control study consisting of 185 patients in Chennai, South India and reported strong association between areca nut

Table 2 Miscellaneous treatment regimens for oral lichen planus

Topical treatments	Systemic treatments	Surgery
Corticosteroids (triamcinolone, fluocinolone acetoneide, fluocinonide, clobetasol, fluticasone propionate, betamethasone sodium phosphate, mometasone furoate)	Corticosteroids	Resection
Cyclosporin	Acitretin	Cryotherapy
Tacrolimus	Azathioprine	Lasers
Pimecrolimus	Basiliximab	(CO ₂ , excimer laser)
Rapamycin (sirolimus)	Cyclosporin	
Retinoids (tretinoin, isotretinoin)	Dapsone	
Aloe vera	Eiconol	
Hyaluronic acid 0.2% gel	Enoxaparin	
	Glycyrrhizin	
	Hydroxychloroquine	
	Interferon alpha	
	Levamisole	
	Mycophenolate mofetil	
	Thalidomide	
	Tetracycline	
	Mesalazine	
	Phenytoin	
	Griseofulvin	

use and OSMF. Mehrotra *et al*^[43] firstly investigated lipid profile in Indian patients with OSMF, and they observed a significant decrease in plasma total cholesterol, high-density lipoprotein cholesterol (HDL) cholesterol and Apo-A1 in patients with OSMF as compared to the controls. Similarly, Kumar *et al*^[44] reported a statistically significant decrease in plasma total cholesterol, LDL and HDL cholesterol in patients with OSMF as compared to controls. Arakeri *et al*^[45,46] observed that the mean concentration of copper in the home drinking water of patients with OSMF was significantly higher than in controls. Patients with OSMF also had a significantly higher copper concentration in serum and saliva, and serum ceruloplasmin than controls^[45,46]. Aggarwal *et al*^[47] reported that the serum beta carotene levels was significantly lower in patients with OSMF than in the controls. From these results, authors suggested that beta carotene plays an important role in the pathogenesis of OSMF and should be treated with a diet rich in beta carotene in order to reduce disease severity and progression towards malignancy^[47]. Higher mast cell density as another possible pathogenic factor in patients with OSMF was suggested by Del Vecchio *et al*^[48].

Symptoms such as burning sensation and/or intolerance to spicy food are the most common symptoms in the initial phase of the disease. Over time, it gradually progresses and fibrosis develops that can affect mouth opening^[37]. Haider *et al*^[49] proposed clinical and functional staging of OSMF, shown in Table 3.

Isaac *et al*^[50] investigated histopathologic features of OSMF and observed some mucosal and submucosal changes. Mucosal changes such as atrophic changes, pigment incontinence, ulceration with granulation tissue, hyperplastic changes, dysplasia, and carcinoma were seen 74.3%, 62.8%, 40%, 25.7%, 8.6% and 0%, respectively. Submucosal changes such as fibrosis, diffuse chronic inflammatory infiltrate, atrophy of minor salivary glands, skeletal muscle atrophy, bandlike infiltrate, edema and

Table 3 Clinical and functional staging

Clinical stage	Functional stage
Faucial bands only	Mouth opening ≥ 20 mm
Faucial and buccal bands	Mouth opening 11-19 mm
Faucial, buccal, and labial bands	Mouth opening ≤ 10 mm

congestion, and vesicle formation were observed 100%, 100%, 85.7%, 57.1%, 45.7%, 22.8%, and 2.8%, respectively^[50].

Three current treatment modalities including surgical, physical, and medical are available for the management of OSMF. Surgical treatments may be used to improve mouth opening and movements. Physical treatment including physical exercise regimen, splints or other mouth opening devices, and microwave diathermy may be useful in some patients with OSMF. There are numerous medical therapy alternatives such as steroids, interferon gamma, placental extracts, immunized milk, pentoxifylline, buflomedil hydrochloride, nyldrin, isoxsuprine, β -carotene, lycopene, vitamins, micronutrients, collagenase, hyaluronidase, chymotrypsin, and aloe vera^[37,51-57].

ACTINIC CHEILITIS

Actinic cheilitis is a potentially malignant disease of the lip caused by exposure solar radiation. It is commonly seen the surface area of the lower lip due to the anatomic proximity. In addition to solar rays, tobacco use, lip irritation, poor oral hygiene, and ill-fitting dentures may play a role in the development of actinic cheilitis. The disease predominantly occurs in men compared to the women^[58]. Martins-Filho *et al*^[59] reported that the prevalence of actinic cheilitis in farmers in a semi-arid area of Brasil was 16.7%.

While actinic cheilitis shows erythema and edema in the early stages of the disease, diffuse scaling, thickened epithelium with small greyish-white plaques (known as *leukoplakia*), inflammatory areas (known as *erythroleukoplakia*), and linear fissures may present in the late stages of the disease^[58]. Malignant transformation rate has been estimated ranging from 1.4% to 36% at an interval of 1 to 30 years^[60]. Diagnosis should be confirmed by biopsy to evaluate the degree of dysplasia. Histopathologically, hyperplasia, acanthosis or atrophy of the epithelium, thickening of the keratin layer, and/or dysplasia, which may range from mild to severe, may be shown. In addition to these epithelial changes, in connective tissue, basophilic degeneration of collagen fibers, known as solar elastosis, is usually detected^[61].

In treatment, 5-fluorouracil, scalpel vermilionectomy, chemical peel, electrosurgery, cryosurgery, CO₂ laser, imiquimod, photodynamic treatment, diclofenac 0.3% gel can be preferred^[58,60,62].

SOME INHERITED CANCER SYNDROMES

In patients with xeroderma pigmentosum and Fanconi's anemia, incidence of oral cancer has increased^[1].

IMMUNODEFICIENCY

In patients with prolonged use of immunosuppressive drugs after solid organ transplants, human immunodeficiency virus-patients, and chronic graft versus host disease after stem cell transplantation are the patients in risk group for oral cancer development^[1].

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Eosinophilic chronic rhinosinusitis in East Asians

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Abstract

Chronic rhinosinusitis (CRS) is a common disease worldwide, with a prevalence rate of 5%-15% in the general population. CRS is currently classified into two types: CRS with and without nasal polyps. CRS may also be divided into eosinophilic CRS (ECRS) and non-ECRS subtypes based on the presence of tissue eosinophilic infiltration or not. There are significant geographic and ethnic differences in the tissue eosinophilic infiltration, which is predominant in Western white patients and less common in East Asians, despite an increasing tendency for its prevalence in East Asia countries. ECRS differs significantly from non-ECRS in clinical characteristics, treatment outcomes and strategies, and underlying pathogenic mechanisms. ECRS commonly demonstrates more severe symptoms, polyp diseases with a higher incidence of bilateral polyps and sinonasal diseases on computed tomography, and the increase in blood eosinophils. ECRS is considered a special and recalcitrant subtype of CRS, commonly with poor treatment outcomes compared to non-ECRS. The differentiation of specific subtypes and clinical features of CRS will be important for developing novel treatment strategies and improving treatment outcomes for individual phenotypes of CRS. This review discusses clinical features, diagnosis, treatment and prognosis of ECRS in East Asians.

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Key words: Chronic rhinosinusitis; Eosinophilic chronic rhinosinusitis; Eosinophils; Chronic rhinosinusitis with nasal polyps; Nasal polyps

Core tip: Chronic rhinosinusitis (CRS) is a common disease and currently classified into two types based on presence or absence of nasal polyps. CRS may also be subtyped into eosinophilic CRS (ECRS) and non-ECRS according to the presence of predominant tissue eosinophilic infiltration or not. ECRS differs significantly from non-ECRS in clinical characteristics, treatment outcomes and strategies, and underlying pathogenic mechanisms. ECRS is considered a special and recalcitrant subtype of CRS. The identification of ECRS is helpful to develop treatment strategies for this CRS subtype. Herein we review the clinical features, diagnosis, treatment and prognosis of ECRS in East Asians.

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INTRODUCTION

Chronic rhinosinusitis (CRS) is one of the most common chronic diseases worldwide, with a prevalence rate of 5%-15% in the general population in Europe and the United States^[1] and 7% in South Korea^[2]. CRS remains a significant public health problem with a considerable socioeconomic burden^[3]. In the current practice guidelines of Europe, the United States and China, CRS is classified into two types based on the presence or absence of nasal polyps: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSSNP)^[1,4,5]. Eosinophilic inflammation is considered a major pathologic hallmark of CRS. Histological studies demonstrate the predominant tissue eosinophilic infiltration with a high proportion of CRS

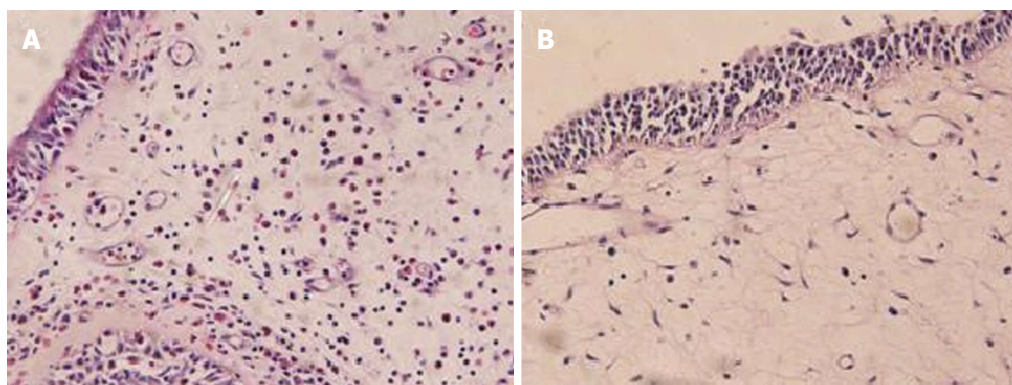


Figure 1 Hematoxylin and eosin staining for nasal polyp tissues. Predominant eosinophil infiltration is showed in the subtype of eosinophilic chronic rhinosinusitis with nasal polyps (A), but other forms of inflammatory cell infiltration in the subtype of non-eosinophilic chronic rhinosinusitis with nasal polyps (B) ($\times 400$).

cases, most prominently with CRSwNP cases^[1]. Thus, CRS may be classified into two subtypes: eosinophilic CRS (ECRS) and non-eosinophilic CRS (NECRS). Similarly, CRSwNP may also be subclassified into ECRSwNP and NECRSwNP^[6-19]. However, the tissue eosinophilic infiltration in CRS shows significant geographic and ethnic differences. Eosinophilic infiltration is predominantly observed in Western white patients with CRS, accounting for more than 80% of CRS cases^[1,4,18], while the eosinophilic phenotype is less than 50% of CRS cases in East Asia countries including Japan^[13-15,20], South Korea^[17,21,22] and China^[11,16,23,24]. However, recent studies indicate an increasing tendency for the prevalence of ECRS in these Asia countries^[12,13,15,20,21,25]. Studies show that ECRS differs significantly from NECRS in clinical characteristics, underlying pathogenic mechanisms, treatment outcomes and strategies^[1,6,10,11,13,15,16,20,26-28]. ECRS is considered a special subtype of CRS^[10,13,15] and also a subtype of recalcitrant CRS, which commonly has worse disease severity^[8,18,19,29] and poorer treatment outcomes^[19,28,30] compared to NECRS. For example, ECRSwNP is refractory to the combined treatments of endoscopic sinus surgery (ESS) and macrolide therapy and shows a strong tendency for recurrence after surgery but responds to systemic steroid therapy^[13-15]. Thus, identifying specific subtypes of CRS and underlying pathogenic mechanisms will be important for developing novel treatment strategies and improving treatment outcomes for individual phenotypes of CRS^[10,29].

ROLES OF EOSINOPHILS IN ECRS

CRS is a heterogeneous disease to which numerous etiologies contributed. Although intensive investigations have been performed, the etiology, pathogenesis and underlying mechanisms of CRS are not fully understood^[1,15,31,32]. The dominant eosinophilic inflammation for CRS indicates that eosinophils play a key role in the pathogenesis of CRS, especially in CRSwNP^[1], although many kinds of other inflammatory cells including neutrophils, mast cells, lymphocytes and plasma cells also have important roles in the pathogenesis of CRS^[27,33] (Figure 1).

Eosinophils develop from CD34⁺ progenitors in the

bone marrow and migrate into the bloodstream, and they are recruited to disease sites by chemokines or cytokines, where eosinophils can perform and participate in a variety of functions, including antigen presentation, cytokine or chemokine production, and secretion of granule mediators^[34-36]. The ability of eosinophils to process and present antigens has been generally underestimated and this function can be added to the growing list of mechanisms by which eosinophils regulate the immune system^[34]. Eosinophils store preformed cytokines in granules that can be released rapidly upon antigenic provocation^[36]. These eosinophil-derived cytokines can be T helper 2 (Th2) cytokines such as interleukin (IL)-13 that act directly on T cells, as well as other inflammatory cytokines that can prime antigen presenting cells and the vascular endothelium to secrete chemokines and cytokines that recruit and activate T cells^[34].

Studies indicate significant roles of T cell regulation in CRS. CRS appears to be a disease mediated by CD4⁺ T cells that can be functionally divided into Th1 or Th2 phenotype based on their patterns of cytokine secretion. It is found that among West white patients with CRS, CRSsNP is characterized by Th1 polarization, whereas CRSwNP by predominant Th2, with high levels of Th2-type cytokines including IL-4, IL-5 and IL-13^[37]. CRSwNP also is characterized by a Th2-driven eosinophilic inflammation in tissue^[37,38]. However, studies suggest that East Asians with CRSwNP present different immunopathologic features compared with West white patients^[17,24,39]. For example, CRSwNP in Chinese demonstrates a Th1/Th17 cell pattern with minor eosinophilic inflammation^[24]. Th2-dominated reactions can only be found in ECRSwNP instead of all CRSwNP cases, suggesting that Th cell responses may exert different impacts on the pathogenesis of ECRSwNP and NECRSwNP^[16,17,24]. There are interactions between T cells and eosinophils. It is conventionally viewed that the T cell–eosinophil interactions are primarily based on the activation of eosinophils by T cells *via* cytokines, but it is suggested that eosinophils also have the capacity to activate T cells to produce cytokines^[40,41]. Eosinophils by secreting specific cytokines or chemokines have a more central role in Th2 responses in CRS^[34].

Table 1 Demographic and clinical characteristics of eosinophilic chronic rhinosinusitis with nasal polyps and non-eosinophilic chronic rhinosinusitis with nasal polyps

	ECRSwNP	NECRSwNP
n (%)	27 (45%)	33 (55%)
Age (yr), mean \pm SD	46.93 \pm 12.35	40.27 \pm 13.47
M/F	20/7	24/9
With AR (%)	74.10%	48.5%
With asthma (%)	18.50%	12.1%
Duration of symptom (yr)	5.50 \pm 3.92	8.55 \pm 6.93
VAS	4.04 \pm 1.01	3.99 \pm 1.09
Score of olfactory dysfunction	5.59 \pm 2.54	5.21 \pm 2.66
Score of polyps	3.59 \pm 1.11 ^b	2.06 \pm 0.82
Incidence of bilateral polyps	92.6% ^b	39.9%
Score of disease on CT	14.42 \pm 3.84 ^b	9.64 \pm 3.37
Serum IgE (kU/L)	236.72 \pm 157.77	167.97 \pm 176.77
Blood eosinophil count ($\times 10^9$ /L)	0.44 \pm 0.24 ^b	0.21 \pm 0.11
Blood eosinophil percentage (%)	6.49 \pm 3.27 ^b	3.42 \pm 1.87
Tissue eosinophil count/HPF	31.56 \pm 21.37 ^b	0.91 \pm 0.80

^b $P < 0.01$ vs NECRSwNP. ECRSwNP: Eosinophilic chronic rhinosinusitis with nasal polyps; NECRSwNP: Non-eosinophilic chronic rhinosinusitis with nasal polyps; M/F: Male/female; AR: Allergic rhinitis; VAS: Visual analogue scale; HPF: High power field; CT: Computed tomography.

CLINICAL FEATURES OF ECRS

Many studies have shown that ECRS differs from NECRS in clinical features^[13,14,15]: (1) ECRS often shows the symptom of olfactory dysfunction in its early stage; (2) ECRS commonly demonstrates multiple and bilateral nasal polyps, with highly viscous mucus secretion, while NECRS mostly with mucopurulent discharge; (3) ECRS tends to have bilateral sinus diseases on sinonasal computed tomography (CT), with a predominant disease in the ethmoid sinus especially in early stage, while NECRS in the maxillary sinus; (4) Co-existence of asthma is more common in ECRS; (5) Most of ECRS cases show the increase of peripheral blood eosinophils; (6) ECRS demonstrates dominant tissue eosinophilic infiltration; (7) In medical treatments, local or systemic steroid therapy is more effective for ECRS compared to macrolide therapy, while macrolide is effective for NECRS; and (8) ECRS shows strong tendency for nasal polyp recurrence after surgery, but systemic steroid is effective for the recurrent nasal polyps.

Symptoms of ECRS

Many studies indicate that ECRS commonly has more severe disease and higher symptom score compared to NECRS^[8,18,19,29]. A recent study shows the mean severity score of symptoms including olfactory dysfunction, nasal obstruction, and nasal discharge in ECRS is significantly higher than that in NECRS^[42]. Previous studies have shown that there is a close correlation between symptoms and tissue eosinophil infiltration in CRS^[18,43]. However, a recent study shows no significant differences in the symptom severities of nasal obstruction, nasal discharge, and facial pain aside from smell dysfunction between ECRS and NECRS cases^[10]. Another study also shows no difference in visual analogue scale (VAS) score or duration of symptoms between ECRSwNP and NECRSwNP

patients^[11], suggesting that the two subtypes may have an equivalent severity of symptoms. Similarly, ECRSwNP and NECRSwNP patients may present with comparable symptom scores^[44]. In our recent study, a significant difference in the mean VAS score of symptoms between the ECRSwNP and NECRSwNP patients was also not found (Table 1).

CRS is one of the most frequent causes of olfactory dysfunction (reduction or loss of smell) and accounts for 21%-25% of cases with smell loss^[45-48]. Meanwhile, olfactory dysfunction affects about 60% of CRS patients^[4]. Olfactory dysfunction is related to the severity of CRS, especially when with nasal polyps^[49]. A study shows that 38% of CRS patients present with olfactory dysfunction, which is affected by nasal polyps, and the prevalence of olfactory dysfunction is 57% in CRSwNP and 13.7% in CRSsNP, respectively^[20]. A recent report indicates that smell dysfunction is a very common symptom in CRSwNP, even accounting for 96.5% of cases^[41]. Olfactory dysfunction is a more predominant and characteristic symptom of ECRS and tends to occur in the early stage of ECRS^[10,13-15,20,50]. This symptom is more severe and common in ECRS compared to NECRS^[42]. A study shows that there is a high prevalence of olfactory dysfunction in ECRS (78.9%) compared to NECRS (25.9%)^[20]. Olfactory dysfunction is reported to be associated with olfactory cleft opacification on CT images^[51]. Nasal polyps occur more commonly in the olfactory cleft in ECRS compared to NECRS^[42]. Edematous swelling or polyposis of the middle turbinate, which is often observed in ECRS patients, increases the opacification of the olfactory cleft and causes olfactory impairments^[13]. Studies indicate that olfaction score is influenced by mucosal eosinophilic infiltration, with lower olfaction score in ECRSwNP as compared to NECRSwNP^[29,52]. A study shows that there are no statistically significant differences in the VAS scores of nasal obstruction, nasal discharge, headache or overall symptoms, but a statistically significant difference is found in relation to problems of smell between the patients with high and low infiltration of eosinophils in the ethmoidal sinus mucosa^[50]. But in our recent study, no statistically significant difference in olfactory dysfunction scores was found between ECRSwNP and NECRSwNP (Table 1). The patients with ECRSwNP seemed to have a shorter duration of symptoms than NECRSwNP patients although this difference was not significant statistically (Table 1).

Polyps in ECRS

ECRS commonly exhibits multiple and bilateral nasal polyps compared to NECRS^[13-15], and the polyps commonly exist in the olfactory cleft^[42]. Although a previous study shows that there is not a significant difference in endoscopic scores of nasal polyps between ECRSwNP and NECRSwNP subtypes^[29], many studies demonstrate that ECRSwNP often present with a higher endoscopic score of nasal polyps compared with NECRSwNP^[10,18]. Our recent study showed that ECRSwNP presented with a higher score of nasal polyps and a higher incidence of

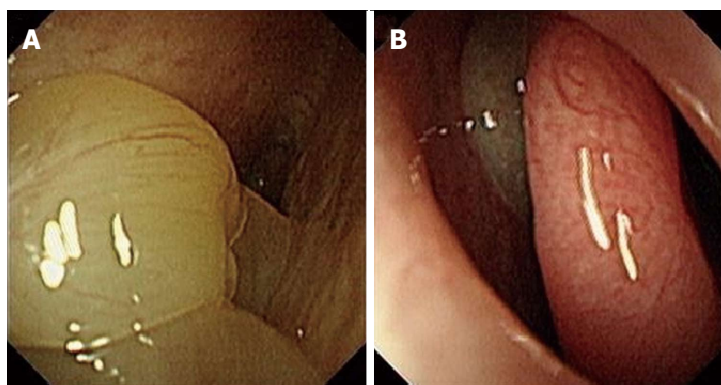


Figure 2 Nasal endoscopic findings. Polyps in eosinophilic chronic rhinosinusitis with nasal polyps (A) and in non-eosinophilic chronic rhinosinusitis with nasal polyps (B).

Table 2 Computed tomography features of eosinophilic chronic rhinosinusitis with nasal polyps and non-eosinophilic chronic rhinosinusitis with nasal polyps

	ECRSwNP (n = 27)	NECRSwNP (n = 33)
Total disease score of sinuses	14.42 ± 3.84 ^b	9.64 ± 3.37
Number of involved sinuses	7.88 ± 1.22 ^b	5.64 ± 1.49
Percentage of involvement in all sinuses	23.1% ^a	3.0%
Incidence of bilateral diseases in individual sinuses		
Frontal	53.8% ^b	12.1%
Sphenoid	38.5% ^a	9.1%
Anterior ethmoid	53.8% ^a	12.1%
Posterior ethmoid	100.0% ^b	75.8%
Maxillary	96.2% ^a	69.7%
OMC	69.2% ^a	36.4%
Score of diseases in individual sinuses		
Frontal	1.81 ± 1.51 ^a	0.88 ± 0.91
Sphenoid	1.23 ± 1.28 ^a	0.55 ± 0.76
Anterior ethmoid	3.27 ± 0.90 ^b	2.27 ± 0.87
Posterior ethmoid	3.04 ± 1.04 ^b	1.52 ± 0.95
Maxillary	2.23 ± 0.43	2.15 ± 0.69
OMC	2.85 ± 1.60	2.27 ± 1.20

^a $P < 0.05$, ^b $P < 0.01$ vs NECRSwNP. Scoring for sinus diseases on computed tomography (CT): 0 = normal, 1 = partial opacification, and 2 = total opacification; these points are applied to individual sinuses on each side; OMC is graded as 0 = not occluded, or 2 = occluded; deriving a maximum score of 12 per side. ECRSwNP: Eosinophilic chronic rhinosinusitis with nasal polyps; NECRSwNP: Non-eosinophilic chronic rhinosinusitis with nasal polyps; OMC: Ostiomeatal complex.

bilateral nasal polyps when compared with NECRSwNP (Table 1 and Figure 2).

In addition, endoscopic examination indicates that most of patients with ECRS demonstrate sinonasal mucus secretion with high viscosity, while NECRS is common with mucopurulent discharge^[13-15]. It was found in our recent study that more than half (55.6%) of 27 ECRSwNP patients showed highly viscous mucus secretion, but less than a third (30.3%) of 33 NECRSwNP patients presented with this condition.

CT findings in ECRS

The Lund-Mackay scoring system is widely used to evaluate the disease severity of CRS on sinonasal CT^[1,4,53,54]. CRSwNP tends to have a higher score of disease on CT compared with CRSsNP^[41]. CT imaging also is a powerful tool to differentiate ECRS from NECRS^[13]. Studies show

that there are significant differences in the disease scores of most sinuses aside from maxillary sinus between ECRS and NECRS^[13,15]. ECRSwNP presents with higher disease scores on CT compared to NECRSwNP^[18,27], although an obvious difference in CT scores between ECRSwNP and NECRSwNP subtypes is not found in some studies^[11,29]. In addition, CT studies show that sinus diseases commonly occur bilaterally in ECRS compared to NECRS^[13-15]. Our recent study showed significant differences in the mean score of total diseases in all sinuses, the mean number of involved sinuses, the percentage of cases with involvement of all sinuses, and the incidence of bilateral diseases in individual sinuses between ECRSwNP and NECRSwNP (Table 2 and Figure 3).

In terms of individual sinuses, ECRS patients especially in their early stages often have predominant diseases in the ethmoid sinuses^[13-15,20,42]. A previous study shows that there is a significant correlation between the severity of eosinophilic infiltration in the ethmoidal mucosa and the disease on CT^[55]. Ethmoidal sinus lesions are readily detected by CT in patients with CRS accompanied by severe eosinophil infiltration^[50]. Involvement of the posterior ethmoid sinus is one of the most apparent differences in CT images between ECRS and NECRS. In the early stage of ECRS, CT images can demonstrate the opacification of the posterior ethmoid sinus^[15]. A study shows that the posterior ethmoid sinus is more commonly involved in ECRSwNP compared to NECRSwNP, whereas both the anterior and posterior ethmoid sinuses are similarly involved in NECRSwNP, and CT score of the posterior ethmoid has a good accuracy as a predictor of ECRSwNP in a Japanese population^[13]. Our recent study showed that ECRSwNP had a higher incidence of bilateral diseases and a higher disease score in the anterior or posterior ethmoid sinus compared to NECRS, but ECRSwNP had similar disease scores in its anterior and posterior ethmoid sinuses, while NECRS showed a higher disease score in the anterior ethmoid sinus compared with the posterior ethmoid sinus (Table 2).

The maxillary sinus is most often involved in CRS. The middle meatus or ostiomeatal complex (OMC) has a fundamental role in the pathogenesis of CRS^[1]. As the drainage from the sinus to the middle meatus or OMC is impaired, the sinus becomes secondarily involved. According to this pathogenesis, sinuses that are most likely

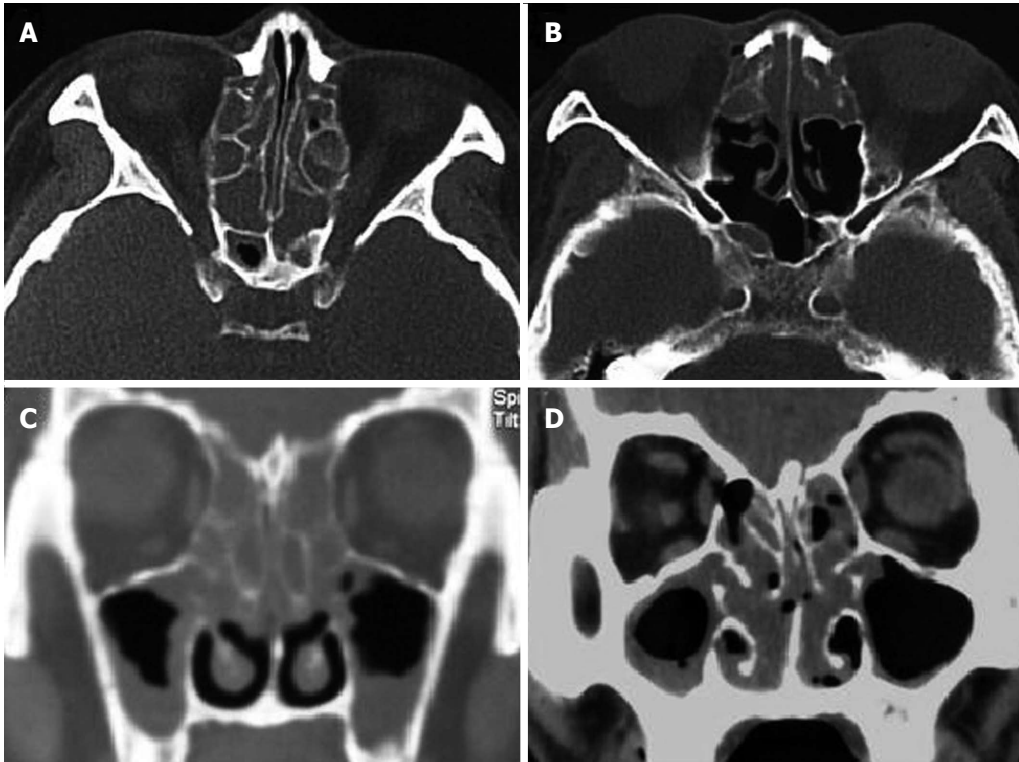


Figure 3 Computed tomography findings. Axial and frontal sections in the subtypes of eosinophilic chronic rhinosinusitis with nasal polyps (ECRSwNP) (A and C) and non-eosinophilic chronic rhinosinusitis with nasal polyps (NECRSwNP) (B and D). Predominant diseases in bilateral anterior and posterior ethmoid sinuses are showed in ECRSwNP, while predominant diseases in anterior ethmoid sinuses in NECRSwNP.

to be affected are the maxillary sinus and anterior ethmoid sinus that connect to the middle meatus or OMC through the small ostia. However, ECRS has predominant disease in the ethmoid sinus, while NECRS in the maxillary sinus^[13-15,20], and the posterior ethmoid sinus that does not directly connect with the middle meatus is involved in similar to the anterior ethmoid sinus even in the early stage for ECRS patients. This suggests that pathological changes in the middle meatus or OMC may be of less importance for the pathogenesis of ECRS, namely, the pathogenesis of ECRS is different from that of NECRS^[15]. A recent study reveals that OMC obstruction is correlated with sinus disease only for patients with CRSsNP but not CRSwNP^[56]. It is thought that ECRS may not be associated with OMC occlusion^[8].

In contrast to NECRS patients who have often a predominant disease in the maxillary sinus, patients with ECRS have commonly a predominant disease in the ethmoid sinus especially in the early stage^[9,13-15,20]. Our recent study showed that ECRSwNP had higher disease scores in frontal, sphenoid, anterior and posterior ethmoid sinuses than NECRSwNP, but there was not a significant difference in maxillary or OMC disease score between ECRSwNP and NECRSwNP (Table 2), which indicated that ECRSwNP had predominant disease in the ethmoid sinus including the anterior and posterior ethmoid sinuses, while NECRSwNP had similar involvement of the anterior ethmoid and maxillary sinuses but with less involvement in the posterior ethmoid sinus.

Co-morbid allergic rhinitis or asthma in ECRS

Inflammation in the upper respiratory tract affects the lower respiratory tract and *vice versa*. The concept of the unified airway is proposed based on evidence from epidemiological, pathophysiological, and treatment outcome studies, indicating the existence of similar inflammatory responses and the shared pathophysiological mechanisms between allergic rhinitis (AR), asthma and CRS^[20,57,58].

Some studies demonstrate that 25%-58% of individuals with CRS have AR^[59,60]. A recent study shows that 67.2% of 418 patients with CRS have AR, and 76.8% of 190 patients with ECRS and 59.2% of 228 patients with NECRS have AR^[42]. However, some studies show that there is not a statistically significant difference in the coexistent rate of AR between ECRSwNP and NECRSwNP^[11,22], and a similar finding was also found in our case cohort (Table 1).

The clinical relationship between CRS and asthma has been known for many years. CRS and asthma coexist often clinically and they share some histopathologic features such as chronic eosinophilic inflammation, epithelial damage, and basement membrane thickening of the airway mucosa^[61]. It is reported that the prevalence of asthma in CRS patients is 20%-50%^[13,18,20,41,42,62,63] and even more than 50%^[61]. However, there is a lower prevalence of asthma (2%-3%) in CRS patients in China compared with the Western population^[64]. This difference may result from distinct immunopathologic characteristics of CRS in Chinese patients, specifically from lower levels of eosinophilic inflammation^[16,24,64-66]. CRS espe-

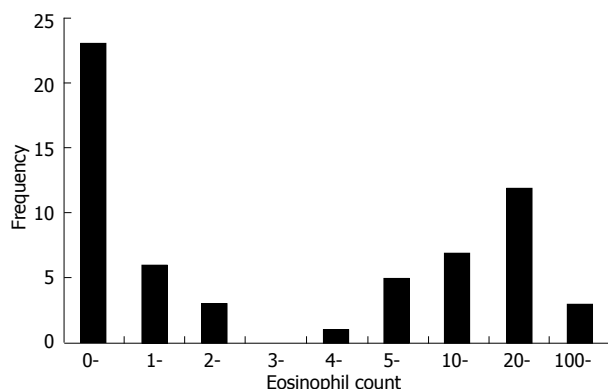


Figure 4 Frequency distribution and range of tissue eosinophil count per high power field for 60 patients with chronic rhinosinusitis with nasal polyps.

cially CRSwNP is commonly associated with asthma^[31]. The association of CRSwNP and asthma is well established, and CRSwNP in white population of Europe and the United States represents often a form of severe and difficult-to-treat eosinophilic airway inflammation, which frequently is linked to co-morbid asthma^[1]. Eosinophilic inflammation is considered a common mechanism in both CRSwNP and asthma^[67]. It is reported that among 2176 cases with CRSwNP, 37.5% present with asthma^[68]. A recent study shows that among 182 patients with CRSwNP, the percentage of patients with asthma is as high as 94%^[69]. Asthma is known to be often concurrent with ECRS^[14,42,70]. A study shows that 34.7% of 190 patients with ECRS, but only 9.6% of 228 patients with NECRS, present the coexistence with asthma^[42]. Co-morbid asthma is one of typical features for ECRS^[13]. Association of ECRSwNP with asthma is widely accepted^[12]. Some authors believe that ECRS and asthma share similar histopathologic features and are the same inflammatory process demonstrating in different sites of the respiratory tract^[61,67].

A study shows that in Chinese patients with CRSwNP, the incidence of asthma (15.9%) in ECRSwNP is higher than that (3.6%) in NECRSwNP^[9]. Another study shows that the prevalence of asthma in ECRSwNP is higher than that in NECRSwNP, but the difference does not reach statistical significance^[11]. And also, there are the studies showing no significant difference in the prevalence of asthma between ECRSwNP and NECRSwNP patients^[11,22], which may be due to the low prevalence of asthma among CRS patients in China^[64]. A statistically significant difference in the incidence of asthma between ECRSwNP and NECRSwNP patients was also not found in our recent study (Table 1). Although asthma is often seen in patients with ECRS, co-morbidity with asthma may not be a diagnostic criterion for ECRS because about half of ECRS cases are not associated with asthma^[15].

IgE and ECRS

CRS is a form of eosinophil-dominated inflammation. Some factors result in local production of IgE, which may contribute to severe eosinophilic inflammation.

There is a significant correlation between the concentration of IgE and the number of eosinophils in nasal polyp tissue^[38]. Some ECRS patients show the elevation of total or specific IgE level^[6,15,44]. ECRSwNP patients demonstrate increased blood IgE levels compared with NECRSwNP^[11,27]. A study shows that the amount of tissue eosinophils in CRSwNP is related to eosinophilia of the peripheral blood, but no significant correlation exists between elevated serum IgE and the increase of tissue or blood eosinophils, indicating that atopic conditions may play a minor role in the pathogenesis of CRSwNP in Koreans^[17]. It is showed that only less than half of CRS patients present with the increased blood IgE and thus eosinophilic inflammation is not likely driven by an IgE mechanism^[61]. A study shows the absence of a significant difference in total serum IgE levels between ECRS and NECRS patients, suggesting that systemic IgE does not greatly contribute to the pathophysiology of ECRS^[13]. Also, a recent study shows that although total serum IgE in ECRS is higher than that in NECRS (120.3 *vs* 48.0 kU/L), the difference is not statistically significant^[10]. Similarly, a significant difference in serum IgE levels between ECRSwNP and NECRSwNP was not found in our recent study (Table 1).

DEFINITION OR DIAGNOSIS OF ECRS

Currently ECRS is determined primarily based on tissue eosinophilic infiltration, but there is not a well-defined criterion of the tissue eosinophilic infiltration for diagnosis of ECRS. In some studies, ECRS including ECRSwNP is defined as tissue eosinophil count per high power field (HPF) more than 5 eosinophils^[18,21,29,64], 10 eosinophils^[8,30], or 20 eosinophils^[9], even more than 100 eosinophils^[27,42], as well as the percentage of eosinophils in tissue-infiltrated inflammatory cells exceeding 5%^[17], 10%^[16,66,71] or 15%^[20]. In our recent study tissue eosinophil count more than 5 eosinophils/HPF was used as a criterion for ECRSwNP based on the frequency of cases with individual eosinophil counts in nasal polyp tissues (Figure 4).

Tissue eosinophilic infiltration, based on which ECRS is determined, is commonly identified after surgery by histopathological examination. Therefore, this approach may be quite unpractical because it is difficult to obtain the diagnostic information before surgery or from the patients treated only with medicines. While peripheral blood eosinophilia has a certain diagnostic value for ECRS^[15], because the close correlation between the number of peripheral blood and tissue-infiltrated eosinophils has been shown in several studies^[9-11,15,17,18,27,42]. It is easy to understand the close association of blood eosinophils with ECRS because the tissue-infiltrated eosinophils are recruited *via* bloodstream to disease sites of ECRS. Many studies have shown that ECRSwNP presents with a significant increase in the peripheral blood eosinophil count or percentage compared to NECRSwNP^[9,11,13,52]. Our recent study also showed the existence of a close correlation between tissue eosinophil count and blood eosino-

Table 3 Diagnostic sensitivity and specificity of blood eosinophil count or percentage for eosinophilic chronic rhinosinusitis

Ref.	Blood eosinophil count				Blood eosinophil percentage			
	AUC	Cutoff value	Sensitivity	Specificity	AUC	Cutoff value	Sensitivity	Specificity
Zuo <i>et al</i> ^[52]	0.873	$0.16 \times 10^9/L$	84.9%	84.4%	0.863	2.05%	89.0%	84.4%
Wang <i>et al</i> ^[9]	-	-	-	-	0.818	5.65%	79.0%	78.2%
Hu <i>et al</i> ^[11]	0.871	$0.22 \times 10^9/L$	74.2%	86.5%	0.864	3.05%	80.3%	75.3%
Sakuma <i>et al</i> ^[13]	-	-	-	-	0.880	6.00%	97.4%	70.7%

ECRS: Eosinophilic chronic rhinosinusitis; AUC: Area under receiver operating characteristic curve.

phil count or percentage in ECRSwNP patients, but not in NECRSwNP patients. Thus, the increased peripheral blood eosinophil count or percentage is considered a good marker or predictor of ECRSwNP^[9,11,13,52]. Some studies show that blood eosinophil count or percentage in ECRS subtype is significantly higher than that in NECRS subtype^[9,11,13,52]. It is found by receiver operating characteristic curve analysis that blood eosinophil count or percentage has high sensitivity and specificity for the diagnosis of ECRS^[9,11,13,52] (Table 3).

Our recent study also showed that there was a statistically significant difference in mean blood eosinophil count or percentage between ECRSwNP and NECRSwNP patients (Table 1). However, it was notable that neither all patients with ECRSwNP had the increased circulating eosinophils nor all patients with NECRSwNP showed a normal level of blood eosinophil count. For example, only 10 of 27 patients with ECRSwNP showed blood eosinophil counts more than normal range and 2 of 33 patients with NECRSwNP had the increase of eosinophil count. Therefore, ECRS or NECRS can not be determined only based on if blood eosinophils increase.

The definition or diagnostic criterion for ECRS is very important since ECRS differs from NECRS in treatment strategy. However, there is not yet a clear definition or diagnostic criterion to differentiate ECRS and NECRS subtypes. Recently, new diagnostic criteria for ECRS have been proposed^[13], in which the diagnosis of ECRS is finally determined by the clinical symptoms, nasal endoscopy, sinonasal CT imaging, peripheral blood test, and histological examination^[13,15].

TREATMENT AND PROGNOSIS FOR ECRS

ESS has been used widely for the treatment of CRS. Outstanding short- and long-term results of ESS in CRS have previously been reported in the literature^[41,68,72-75]. The impact of ESS on the improvement in CRS-related symptoms postoperatively is remarkable. However, some of CRS patients are inadequately controlled despite receiving combination of maximal medical therapy and ESS^[1]. A wide variety of factors contribute to poor disease control, including patient-related factors such as ECRS^[76]. It is believed that NECRS can be relatively well controlled with a combination of ESS and macrolide therapy, whereas ECRS is unresponsive to macrolide therapy^[13]. Many studies indicate that ECRS commonly has poorer

treatment outcomes compared to NECRS^[14,19,28,30,76,77]. For example, ECRSwNP is refractory to the combined treatment of ESS and macrolide therapy and shows a strong tendency for recurrence after surgery^[13-15,27].

However, a recent study suggests that eosinophilic inflammation in CRS may not be related to the surgical outcome in South Koreans^[22]. Another study also shows that the presence or absence of tissue eosinophilic infiltration does not impact significantly on the time interval to revision surgery^[78]. Our recent study showed that in terms of the short-term efficacy of ESS in CRSwNP, both ECRSwNP and NECRSwNP patients had significant improvement in symptoms aside from smell dysfunction at one-week follow-up after ESS, but there was no significant difference in symptom improvement between the two subgroups.

CONCLUSION

In conclusion, CRS can be subclassified into two subtypes: ECRS and NECRS. The prevalence of ECRS is increasing in East Asians in the recent years. ECRS differs from NECRS in clinical features and treatment outcomes; however, there is not yet a universally accepted definition or diagnostic criterion for ECRS, and also the underlying pathogenic mechanisms of ECRS are not well-understood. Identification of ECRS subtypes and underlying pathogenic mechanisms is key to developing treatment strategies for the phenotypes of CRS.

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Spectrum of magnetic resonance imaging findings in congenital lumbar spinal stenosis

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Abstract

AIM: To investigate whether congenital lumbar spinal stenosis (CLSS) is associated with a specific degenerative changes of the lumbar spine.

METHODS: The lumbar spine magnetic resonance imaging studies of 52 subjects with CLSS and 48 control subjects were retrospectively evaluated. In each examination, the five lumbar levels were assessed for the presence or absence of circumferential or shallow annular bulges, annular tears, anterior or posterior disc herniations, epidural lipomatosis, Schmorl's nodes, spondylolisthesis, pars defects, and stress reactions of the posterior vertebral elements.

RESULTS: Compared to control individuals, subjects with CLSS exhibited increased incidence of circumferential and shallow annular bulges, annular tears, disc

herniations and spondylolisthesis ($P < 0.05$).

CONCLUSION: CLSS is associated with increased incidence of degenerative changes in specific osseous and soft-tissue elements of the lumbar spine.

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Key words: Congenital lumbar spinal stenosis; Magnetic resonance imaging; Imaging findings; Degenerative changes; Low back pain

Core tip: Congenital lumbar spinal stenosis is associated with increased incidence of degenerative changes in specific osseous and soft-tissue elements of the lumbar spine. Describing the spectrum of the respective imaging findings, this article can assist radiologists in providing more detailed magnetic resonance imaging reports.

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INTRODUCTION

Since the first reports describing congenital (or developmental) lumbar spinal stenosis (CLSS), the clinical implications of this entity have been the subject of several scientific studies^[1-3]. Based on the original etiological classification described by Arnoldi *et al*^[4], CLSS is a developmental narrowing of the spinal canal, which is secondary to a bone dysplasia. Radiographically, the respective subjects have a shorter pedicular length and as a result a smaller cross-sectional spinal canal area^[5]. In

usual practice, the above subjects tend to present clinically in the fourth or fifth decade of life with various neurogenic complications and relatively few radiographically evident degenerative spondylotic changes^[5]. Except for scattered articles reporting radiographic and cross-sectional measurements to define CLSS, there have been no studies evaluating whether the entity is associated with degenerative changes in specific osseous and soft-tissue elements of the lumbar spine. In our practice with magnetic resonance imaging (MRI) studies of the lumbar spine, we have experienced that individuals with CLSS tend to exhibit an increased incidence of specific imaging features, including foraminal disc protrusions, as well as a particular type of annular disc bulge, in which the posterior concavity of the intervertebral discs is preserved. To validate our observations and potentially extend our understanding of CLSS from an imaging point of view, we investigated the association between CLSS and degenerative changes in various osseous and soft-tissue elements of the lumbar spine.

MATERIALS AND METHODS

Study population

Institutional review board approval was granted and informed consent was waived for this HIPAA-compliant study. The MRI database of our institution was searched for examinations of the lumbar spine performed in adults of less than or equal to 50 years of age, an age limit arbitrarily employed to limit the inclusion of subjects with age-related degenerative spine changes. Two radiologists with 16 (A.C.) and 6 (T.S.) years of radiology experience, respectively, who were blinded to the original reports of the MRI studies, evaluated the examinations in consensus on a picture archiving and communication system workstation (Ultravision, Emageon, AL, United States). All examinations were performed on 1.5T or 3.0T MR scanners and included axial and sagittal T1- and T2-weighted images, as well as sagittal STIR images of the lumbar spine.

In each study, the T2-weighted images were used to calculate the mid-sagittal spinal canal diameter and mid-sagittal thecal sac diameter at the mid-vertebral levels of all five lumbar vertebrae. Subjects with mid-sagittal spinal canal diameter of less than 14 mm on at least one level were considered as having CLSS and were included in the study group, whereas subjects with mid-sagittal spinal canal diameter of equal or greater than 14 mm at all five levels were included in the control group. Examinations were consecutively evaluated until a study group of 52 subjects and a control group of 48 subjects were formed. Patients with achondroplasia or a known history of spinal surgery, trauma, infection and/or tumor were excluded.

Image analysis

In each study, the average value of mid-sagittal thecal sac diameter was calculated, and thereafter, the intervertebral levels from L1-L2 to L5-S1 were evaluated for the presence or absence of (1) circumferential annular bulge,

defined as generalized extension of greater than 50% of the outer boundary of the intervertebral disc beyond the border of the adjacent bone, with loss of posterior disc concavity; (2) shallow annular bulge, defined as the extension of the intervertebral disc by greater than 50% from the outer boundary of the adjacent bone, with preservation of the posterior disc concavity; (3) annular tear(s), defined as focal area(s) of increased signal intensity within the outer layer of the intervertebral disc on fluid-sensitive images; (4) uni- or bilateral foraminal disc herniation(s), defined as extension(s) of less than 50% of the outer boundary of the intervertebral disc beyond the border of the adjacent bone, centered on one or both foramina; (5) central or paracentral disc herniation, defined as extension of less than 50% of the outer boundary of the intervertebral disc beyond the border of the adjacent bone, protruding centrally or subarticularly within the spinal canal, respectively; (6) epidural lipomatosis, registered when the epidural adipose tissue assumed an anteriorly convex border and a thickness of greater than 7 mm^[6]; (7) Schmorl's node(s); (8) spondylolisthesis (antero- or retrolisthesis), registered when the vertebral body exhibited anterior or posterior displacement of equal or greater than 1 mm over the vertebral body below; (9) uni- or bilateral pars defect(s); (10) anterior disc herniation; and (11) stress reaction (increased signal intensity on fluid-sensitive images) of the posterior vertebral elements. For each of the above features, the total incidence observed throughout the five intervertebral levels was documented for each subject.

Statistical analysis

For all evaluated quantitative parameters, the difference between the study and control groups was assessed using Student's *t* test, whereas sex distribution was evaluated using χ^2 test. A probability level of 0.05 was accepted as statistically significant. All data were stored on a spreadsheet (Excel 2010, Microsoft, Seattle, WA, United States) and analysis was performed using a commercially available statistical package (MedCalc 8.0, Mariakerke, Belgium).

RESULTS

Table 1 summarizes the demographics of the study population, the incidences of the features evaluated, as well as the results of the various statistical comparisons between the study and control groups. The two groups were similar in terms of age and sex distribution. Subjects with CLSS exhibited increased incidence of circumferential and shallow annular bulges, foraminal and anterior disc herniations, annular tears, and spondylolisthesis. There was no difference between the two groups regarding the incidences of central and paracentral disc herniations, epidural lipomatosis, Schmorl's nodes, pars defects, and stress reaction of the posterior vertebral elements.

DISCUSSION

CLSS has been attributed to an abnormal anatomic de-

Table 1 Demographics of the 100 patients of the study along with the imaging features which were evaluated on the respective magnetic resonance imaging studies

Parameter	Subjects with CLSS	Control subjects	P
Subjects	52	48	-
Age	38 ± 10	38 ± 8	0.4930
Sex (males/females)	28/24	22/26	0.2742
Average mid-sagittal thecal sac diameter	1.31 ± 0.13	1.51 ± 0.18	-
Circumferential annular bulges	59 (1.13 ± 0.95)	35 (0.73 ± 0.79)	0.0116 ¹
Shallow annular bulges	80 (1.54 ± 1.06)	47 (0.98 ± 0.93)	0.0031 ¹
Foraminal disc herniations	31 (0.60 ± 0.82)	13 (0.27 ± 0.54)	0.0111 ¹
Central/paracentral disc herniations	22 (0.42 ± 0.70)	15 (0.31 ± 0.55)	0.1917
Epidural lipomatosis	33 (0.63 ± 1.09)	17 (0.35 ± 0.76)	0.0701
Schmorl's nodes	24 (0.46 ± 1.00)	13 (0.27 ± 0.68)	0.1352
Spondylolisthesis	53 (1.02 ± 0.96)	29 (0.60 ± 0.71)	0.0081 ¹
Pars defects	0 (0.00 ± 0.00)	2 (0.04 ± 0.20)	0.0699
Annular tears	56 (1.08 ± 1.01)	25 (0.52 ± 0.80)	0.0004 ¹
Anterior disc herniation	63 (1.21 ± 1.16)	25 (1.51 ± 0.18)	< 0.0001 ¹
Posterior elements stress reaction	4 (0.08 ± 0.33)	2 (0.04 ± 0.20)	0.2644

Subjects and sex are expressed as number of cases, age as yr ± SD, and spinal and thecal sac diameters as average value in cm ± SD. All imaging parameters are presented as total incidence (average incidence ± SD). Features marked with an asterisk (*) indicate significant difference between the two groups. CLSS: Congenital lumbar spinal stenosis.



Figure 1 Mid-sagittal T2-weighted (3230, 120) image of the lumbar spine in a 50-year-old male with congenital lumbar spinal stenosis. The lumbar spine shows loss of the lordotic curve, multilevel spondylolisthesis, and degenerative disc disease manifested as loss of disc height, circumferential disc bulges, anterior disc herniations, Schmorl's nodes and a central disc protrusion. In this subject, the mid-sagittal spinal canal diameter ranged from 1.45 cm at the L1 level to 1.03 cm at L4 level. The average mid-spinal canal diameter was 1.26 cm.

velopment of the spinal canal. The etiology of the entity is unknown, except from some cases which are induced by achondroplasia^[5,7]. CLSS differs from degenerative lumbar spinal stenosis in that the spinal canal stenosis is not limited to one or two intervertebral levels, but is uniformly distributed throughout the lumbar spine (Figure 1). As a result, the surgical treatment of CLSS commonly necessitates multi-level intervention, as opposed to degenerative lumbar spinal stenosis, which requires more focal procedures^[5]. Subjects with CLSS are vulnerable to even minimal degenerative changes that compromise the already narrowed spinal canal, and tend to experience symptoms in the fourth and fifth decades of life, as opposed to patients with degenerative lumbar spinal stenosis, who demonstrate symptoms primarily after the sixth decade of life^[5,7].

Although the incidence of CLSS in the general popu-

lation is unknown and probably varies among different races and ethnic groups, MRI readers commonly encounter this entity in studies of the lumbar spine. CLSS has been described as early as the 1950s^[3], however the imaging evaluation of this entity has been limited to delineating radiographic and cross-sectional criteria for its definition, and reporting limited degenerative spondylotic changes as a typical radiographic feature of the respective subjects. The definition of CLSS is not uniform across authors, with studies suggesting cut-off values of mid-sagittal spinal canal diameter varying between 10 and 17 mm, and not clarifying whether spinal canal narrowing needs to be documented on at least one or more spinal levels^[4,7-10]. Some authors consider the cross-sectional area of the spinal canal as the criterion to define CLSS, a measurement, probably more accurate, but also time-consuming and impractical for everyday use. In addition, all previous studies have been limited in the vague description of osseous degenerative changes, and mostly using radiographs^[1-3,5,7]. This study focused on specific osseous and soft tissue elements of the lumbar spine and employed a mid-sagittal spinal canal diameter of less than 14 mm on at least one mid-vertebral level to define CLSS. The latter value “summates” previous reports, has been illustrated in a large previous study by Singh *et al*^[5] and is the one used by radiologists and orthopaedic surgeons in our institution. A recent study which compared subjects with and without CLSS by means of MRI and anteroposterior radiographs of the lumbar spine, reported that, in the CLSS cohort, global pathology and multilevel involvement with L3, L4, and L5 segments were involved more commonly and severely, whereas severe stenosis, at L1, L2, and S1 occurred infrequently. The authors also described three spinal canal morphologies in the CLSS group: (1) “flattened” canal with predominantly reduced spinal canal AP diameter; (2) spinal canal with predominantly reduced interlaminar angle; and (3) global reduction of all canal parameters^[11].

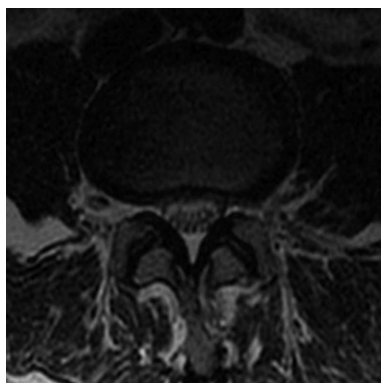


Figure 2 Axial T2-weighted (5000, 102) images of the lumbar spine in a 48-year-old female with congenital lumbar spinal stenosis exhibits a shallow annular disc bulge.

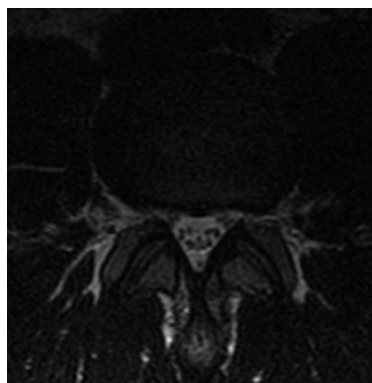


Figure 3 Axial T2-weighted (3516, 115) image of the lumbar spine in a 29-year-old male with congenital lumbar spinal stenosis demonstrates a circumferential disc bulge with a superimposed left foraminal disc protrusion.

We found that young and middle-aged individuals with CLSS demonstrate increased incidence of degenerative changes in the specific osseous and soft-tissue elements of the lumbar spine. In particular, these subjects exhibit increased incidence of “shallow annular bulges”, a term which has not been previously established, but has been used in our practice to describe a particular type of disc bulge, which involves greater than 50% of the disc boundary, but does not meet the strict definition of circumferential bulge, in which a loss of the posterior disc concavity is also present (Figure 2). Shallow annular bulges preferentially narrow the neural foramina, similar to foraminal herniations. It may be speculated that, in CLSS, the thecal sac becomes less pliable due to the uniformly narrowed spinal canal, as well as less compressible due to the opposing intrinsic pressure of the cerebrospinal fluid. As a result, the thecal sac demonstrates increased resistance against posterior disc bulges or herniations in the initial stages of degenerative disc disease. In the above setting, a degenerated disc, acquiring the path of least resistance, tends to project into the foramina rather than the spinal canal. Consequently, the disc potentially maintains its posterior concavity and a shallow annular bulge is established. The above hypothesis could also explain the increased incidence of foraminal protrusions and anterior disc herniations in CLSS. The spectrum of findings is completed with increased incidence of circumferential annular bulges and spondylolisthesis (Figure 3).

Knowledge of the spectrum of MRI findings in CLSS could not only extend our understanding of the latter entity, but also assist radiologists in providing more detailed lumbar spine MRI reports. In most situations, radiologists begin the assessment of lumbar spine MRI studies from a quick evaluation of the mid-sagittal image, therefore establishing CLSS could alert readers for the presence of the aforementioned features.

This study has certain limitations. First, all studied subjects reported back pain, therefore the incidence of degenerative disc disease was probably high in this biased group, and may have affected the results. Second, all MRI examinations were evaluated in consensus by the two readers, therefore the inter-observer variability could

not be estimated. Third, due to the absence of a widely accepted cut-off value of mid-sagittal canal diameter to define CLSS, we used a cut-off value that “summates” previous reports, and probably provides a good trade-off between specificity and sensitivity for detecting CLSS. However, the use of an absolute dividing line between subjects with and without CLSS is probably arbitrary, and a continuous zone between the two groups most likely exists.

In conclusion, CLSS is associated with early development of degenerative changes in specific osseous and soft-tissue elements of the lumbar spine, which could reflect altered spinal biomechanics. The spectrum of imaging findings includes the shallow annular bulge, in which the posterior concavity of the intervertebral disc is preserved. Knowledge of the above spectrum could extend our understanding of CLSS, and assist radiologists in providing more detailed lumbar spine MRI reports.

COMMENTS

Background

Congenital lumbar spinal stenosis (CLSS) is a developmental narrowing of the spinal canal, which is associated with early neurogenic complications and relatively few radiographically evident degenerative spondylotic changes. This article investigates the association between CLSS and degenerative changes in various osseous and soft-tissue elements of the lumbar spine.

Research frontiers

Evaluation of subjects with CLSS by means of magnetic resonance imaging to investigate the spectrum of early degenerative changes of the lumbar spine in the respective entity.

Innovations and breakthroughs

This is the first article to describe the spectrum of early degenerative changes of the lumbar spine in subjects with CLSS.

Applications

Providing knowledge of the spectrum of early degenerative changes of the lumbar spine in subjects with CLSS this article extends our understanding of the entity and may assist radiologists in providing more detailed MRI reports.

Peer review

This is a very interesting article describing uncertain entity as congenital lumbar stenosis is.

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Appendiceal Crohn's disease clinically presenting as acute appendicitis

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Abstract

AIM: To determine the incidence of appendiceal Crohn's disease (CD) and to summarize the characteristic histologic features of appendiceal CD.

METHODS: We reviewed the pathology files of 2179 appendectomy specimens from January 2007 to May 2013. The computer-assisted retrieval search facility was utilized to collect specimens. We selected those cases that were diagnosed as CD or chronic granulomatous inflammation and defined the final diagnosis according to the histologic findings of CD, including transmural lymphocytic inflammation, non-caseating epithelioid granulomas, thickening of the appendiceal wall secondary to hypertrophy of muscularis mucosa, mucosal ulceration with crypt abscesses, mucosal fissures, and fistula formation.

RESULTS: We found 12 cases (7 male and 5 female patients, with an average age of 29.8 years) of appendiceal CD. The incidence of appendiceal CD was 0.55%. The chief complaints were right lower quadrant pain, abdominal pain, lower abdominal pain, and diarrhea. The duration of symptom varied from 2 d to 5 mo.

The histologic review revealed appendiceal wall thickening in 11 cases (92%), transmural inflammation in all cases (100%), lymphoid aggregates in all cases (100%), epithelioid granulomas in all cases (100%), mucosal ulceration in 11 cases (92%), crypt abscesses in 5 cases (42%), perforation in 2 cases (17%), muscular hypertrophy in 1 case (8%), neural hyperplasia in 5 cases (42%), and perpendicular serosal fibrosis in 8 cases (67%).

CONCLUSION: A typical and protracted clinical course, unusual gross features of the appendix and the characteristic histologic features are a clue in the diagnosis of appendiceal CD.

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Key words: Appendix; Appendectomy; Acute appendicitis; Crohn's disease; Prognosis

Core tip: Appendiceal Crohn's disease (CD) is relatively rare and is indistinguishable from acute appendicitis. Appendiceal CD shows a favorable clinical outcome with a low recurrence rate. The differential diagnosis includes intestinal tuberculosis, foreign body reaction, diverticulitis of the appendix, sarcoidosis, actinomycosis, and *Yersinia* infection. Atypical and protracted clinical course, unusual gross features of the appendix and the characteristic histologic features are a clue in the diagnosis of appendiceal CD.

Han H, Kim H, Rehman A, Jang SM, Paik SS. Appendiceal Crohn's disease clinically presenting as acute appendicitis. *World J Clin Cases* 2014; 2(12): 888-892 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i12/888.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i12.888>

INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory bowel

disorder characterized by a transmural inflammatory reaction and non-caseating small granulomas and may involve all parts of the gastrointestinal (GI) tract from the mouth to the anus^[1-7]. The most common sites of involvement are the ileum and colon^[8]. Appendiceal CD is a rare disease but has been well summarized in the various reports^[9-14]. The incidence of appendicitis with granulomatous reaction varies from 0.1% to 2.0%^[15]. Since Meyerding *et al*^[16] had reported an interesting case of appendiceal CD without demonstrable involvement of the adjacent GI tract in 1953, many additional cases of appendiceal CD have been demonstrated in the literature to date.

The purpose of this retrospective review study was to determine the exact incidence of appendiceal CD in patients who underwent appendectomy and to summarize the common characteristic histologic findings along with a review of the literature.

MATERIALS AND METHODS

Ethics

The materials used in our study are human appendix tissue samples, which are products of surgical procedures. Our study contains no private information relating to the patients, and so ensures their anonymity. Therefore, our study has no problems in causing any ethical issue or encroachment of human rights.

Patient tissue

A retrospective review of 2179 appendectomy specimens from January 2007 to May 2013 was conducted. All patients underwent appendectomy at the Hanyang University Hospital (Seoul, South Korea). The computer-assisted retrieval search facility was utilized to collect appendectomy specimens. Appendices resected for acute appendicitis and those removed as a part of right hemicolectomy and gynecology procedures were collected and reviewed. We selected those cases that were diagnosed as CD or chronic granulomatous inflammation and defined the final diagnosis according to the common histologic findings of CD, including transmural lymphocytic inflammation, non-caseating small epithelioid granulomas, thickening of the appendiceal wall secondary to hypertrophy of muscularis mucosa, mucosal ulceration with crypt abscesses, mucosal fissures, and fistula formation. No evidence of parasitic, fungal and mycobacterial disease, foreign body, or systemic sarcoidosis was found in any patient. The clinical information including age, gender, clinical data, and data about the surgical procedure for each case as well as follow-up data including colonoscopic evaluation was collected. The special staining technique such as Ziehl-Neelsen staining and special molecular technique such as tuberculosis polymerase chain reaction (Tb-PCR) were performed to rule out *Mycobacterium tuberculosis*.

RESULTS

Out of these 2179 appendectomy specimens, 12 cases

(0.55%) were classified as appendiceal CD. The clinicopathologic characteristics of the appendiceal CD patients are summarized in Tables 1 and 2. Out of these 12 patients, there were 7 male and 5 female patients. The age of patients ranged from 11 to 51 years (average age of 29.8 years). The chief complaints of patients were right lower quadrant (RLQ) pain, abdominal pain, lower abdominal pain, and diarrhea. The duration of symptom with which patients presented varied from 2 d to 5 mo. There was no systemic clinical manifestation such as arthralgia, uveitis, or arthritis. No history of tuberculosis of any organ was found in these patients. There was also no clinical evidence of systemic sarcoidosis. The initial clinical impression was acute appendicitis in all of these 12 patients along with perforation in 2 among these 12 patients. All patients underwent appendectomy. The final pathologic report was CD in all of these 12 cases. All cases showed a negative result for *Mycobacterium tuberculosis* in Ziehl-Neelsen staining and Tb-PCR. The histologic review of these 12 cases revealed appendiceal wall thickening in 11 cases (92%), transmural inflammation in all cases (100%), lymphoid aggregates in all cases (100%), epithelioid granulomas in all cases (100%), mucosal ulceration in 11 cases (92%), crypt abscesses in 5 cases (42%), perforation with abscess formation in 2 cases (17%), muscular hypertrophy in 1 case (8%), neural hyperplasia in 5 cases (42%), and perpendicular serosal fibrosis in 8 cases (67%). The representative microphotographs are shown in Figure 1. There is no evidence of disease recurrence in these 12 patients to date.

DISCUSSION

Crohn first described that CD stops at the ileocecal valve with sparing of the colon and appendix. However, this theory was disproved as patients with CD often have involvement of the colon and appendix^[5]. The first isolated appendiceal CD was reported by Meyerding *et al*^[16] in 1953. Since Meyerding *et al*^[16] had reported a case of CD arising in the appendix, many case reports and some collective reviews have been reported in the literature. The incidence of appendiceal CD is variable^[17-21]. Prieto-Nieto *et al*^[4] described that approximately 0.2% of patients (10 out of 4468 appendectomies performed during 20 years) had appendiceal CD. In our review, 12 cases (0.55%) out of 2179 appendectomy specimens were revealed as appendiceal CD.

Appendiceal CD is usually found among young patients, however, it can occur at any age^[3,12]. Yang *et al*^[14] described the age with onset of disease in 14 patients with appendiceal CD, ranged from 10 to 45 years (average age of 21.1 years). Prieto-Nieto *et al*^[4] reported the disease onset-age in 10 patients with appendiceal CD, ranged from 10 to 33 years (average age of 29 years). The difference in incidence of disease in males and females has been reported, with male predominance^[4,14]. In our study, the age ranged from 11 to 51 years, with an average age of 29.8 years. Among 12 patients, 7 were male, reflecting more male patients with the disease described

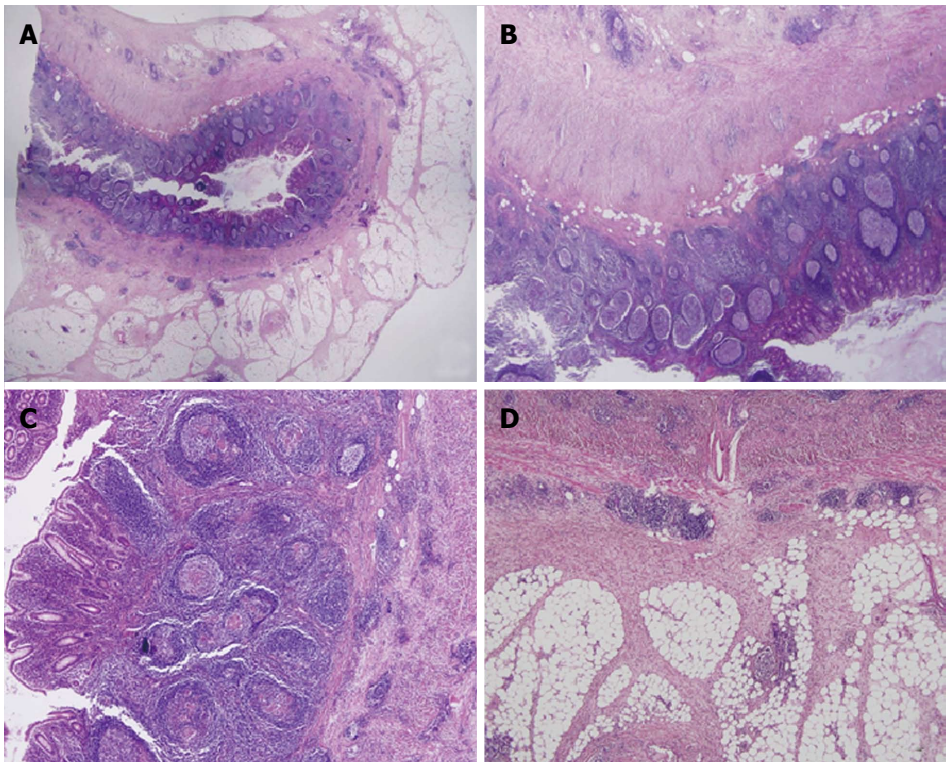


Figure 1 Appendiceal Crohn's disease. A: The appendix with Crohn's disease shows transmural inflammation with markedly thickened wall; B: There is a prominent lymphoid hyperplasia in the mucosa and serosa; C: The mucosa shows many small non-caseating granulomas; D: The serosa shows creeping fat with perpendicular thick fibrous bands.

Table 1 Summary of the patients with appendiceal Crohn's disease							
Case No.	Sex	Age (yr)	c/c	SD	Clinical impressions	AFB	Tb-PCR
1	F	30	RLQ pain	None	Acute appendicitis	Negative	Negative
2	M	38	RLQ pain	14 d	Acute appendicitis	Negative	Negative
3	M	26	RLQ pain	None	Acute appendicitis	Negative	Negative
4	M	28	RLQ pain	7 d	Acute appendicitis	Negative	Negative
5	F	25	RLQ pain	14 d	Acute appendicitis	Negative	Negative
6	F	29	RLQ pain	7 d	Acute appendicitis	Negative	Negative
7	M	51	RLQ pain	5 mo	Acute appendicitis, perforation	Negative	Negative
8	F	49	RLQ pain	8 d	Acute appendicitis	Negative	Negative
9	M	30	Abdominal pain	10 d	Acute appendicitis, perforation	Negative	Negative
10	M	23	Lower abdominal pain	2 d	Acute appendicitis	Negative	Negative
11	M	18	Lower abdominal pain, diarrhea	3 d	Acute appendicitis	Negative	Negative
12	F	11	RLQ pain	7 d	Acute appendicitis	Negative	Negative

F: Female; M: Male; c/c: Chief complaint; RLQ: Right lower quadrant; SD: Symptom duration; AFB: Acid-fast bacillus; Tb-PCR: Tuberculosis polymerase chain reaction.

previously.

The clinical presentation of appendiceal CD is variable. The most common presenting symptom is acute lower abdominal pain especially in the RLQ, which is very similar to the lower abdominal pain presented in patients with acute appendicitis^[4,13,22]. Approximately 25% of appendiceal CD patients show chronic abdominal pain in the right lower abdomen^[13]. The symptoms may be more protracted or recurrent than in the usual case of acute suppurative appendicitis. Appendiceal CD should be suspected when the patients show atypical or protracted unusual clinical course^[2,13]. In our study, most patients presented with the pain in the RLQ. The initial clinical impression was acute appendicitis in all 12 patients. Most patients had symptoms for two or more days, and 8 patients (67%) presented with these symptoms for over a week.

Appendiceal CD usually shows an enlarged appendix with marked thickening of the appendiceal wall and fibrous adhesion to the periappendiceal soft tissue^[2,22,23]. Microscopically, the histologic features are characterized by transmural chronic inflammation with marked fibrous thickening of the wall, lymphoid aggregates, small non-caseating granulomas, ulcerative mucosal change, crypt abscesses, muscular hypertrophy, and neural hyperplasia^[13,24-26]. In our study, the features were similar to the

Table 2 Summary of histologic features of appendiceal Crohn's disease

Histologic features	Number of cases	%
Wall thickening	11/12	92
Transmural inflammation	12/12	100
Lymphoid aggregates	12/12	100
Epithelioid granulomas	12/12	100
Mucosal ulceration	11/12	92
Crypt abscess	5/12	42
Perforation	2/12	17
Muscular hypertrophy	1/12	8
Neural hyperplasia	5/12	42
Perpendicular serosal fibrosis	8/12	67

previously described histologic characteristics. Interestingly, we found that appendiceal CD had the characteristic perpendicular serosal fibrous band formation in 8 out of 12 cases.

The differential diagnosis includes intestinal tuberculosis, foreign body reaction, diverticulitis of the appendix, sarcoidosis, actinomycosis, and *Yersinia* infection^[10,13,22,24,25]. Appendiceal tuberculosis results in the formation of epithelioid granulomas, however, the granulomas in tuberculosis are larger with a central caseous necrosis and less discrete than those in Crohn's disease^[10,27-29]. If a foreign body is present, histologic examination should reveal the offending material and diverticular disease may be excluded via careful examination^[14,27]. Intestinal sarcoidosis is extremely rare and does not occur as an isolated finding^[13,30]. Actinomycosis also results in a vague granulomatous tissue reaction, however, actinomycosis shows neutrophilic abscess formation with floating bacterial colonies (sulphur granules)^[31-34]. *Yersinia* infection results in necrotizing granulomatous reaction in the appendiceal mucosa or submucosa and shows microabscess formation^[35,36].

The treatment of choice for appendiceal CD is appendectomy^[30]. Appendiceal CD shows lower recurrence rate compared with CD arising in other parts of the intestine^[25]. The prognosis of appendiceal CD seems to be much better than that of CD arising in the small or large bowel^[14].

In conclusion, we described the incidence of appendiceal CD in patients who underwent appendectomy and summarized the common characteristic histologic findings along with a review of the literature. Atypical and protracted clinical course, unusual gross features of the appendix and the characteristic features are a clue in the diagnosis of appendiceal CD.

COMMENTS

Background

Appendiceal Crohn's disease (CD) is a rare disease. Since Meyerding *et al* had reported an interesting case of appendiceal CD without demonstrable involvement of the adjacent gastrointestinal tract in 1953, many additional cases of appendiceal CD have been demonstrated in the literature to date.

Research frontiers

The incidence of appendicitis with granulomatous reaction varies from 0.1% to

2.0%. The incidence of appendiceal CD is variable. The purpose of this study was to determine the exact incidence of appendiceal CD in patients who underwent appendectomy and to summarize the common characteristic histologic findings along with a review of the literature.

Innovations and breakthroughs

The histologic features are characterized by transmural chronic inflammation with marked fibrous thickening of the wall, lymphoid aggregates, small non-caseating granulomas, ulcerative mucosal change, crypt abscesses, muscular hypertrophy, and neural hyperplasia. In this study, the features were similar to the previously described histologic characteristics. However, the authors found that appendiceal CD had the characteristic perpendicular serosal fibrous band formation in 8 out of 12 cases.

Applications

With the characteristic clinical presentation and the typical pathologic findings, the clinicians and pathologists can consider the possibility of appendiceal CD. Atypical and protracted clinical course, unusual gross features of the appendix and the characteristic histologic features are a clue in the diagnosis of appendiceal CD.

Terminology

CD is a chronic inflammatory bowel disorder characterized by a transmural inflammatory reaction and non-caseating small granulomas and may involve all parts of the gastrointestinal tract from the mouth to the anus.

Peer review

The authors described appendiceal CD clinically presenting as acute appendicitis. This is an interesting review and CD in appendix is a rare condition. Whenever it is encountered, the surgeon must know what to do and be aware of its prognosis. This paper will lead surgeons to this condition.

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Tranexamic acid for the management of uterine fibroid tumors: A systematic review of the current evidence

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Key words: Tranexamic acid; Uterine fibroids; Fibrinolysis; Menorrhagia; Myomectomy

Core tip: Uterine fibroid tumors are the most common gynecologic causes for menorrhagia. Tranexamic acid is a safe non-hormonal medication that significantly reduces abnormal menstrual bleeding. We conducted a systematic review of the contemporary evidence on the administration and efficacy of tranexamic acid in patients with menorrhagia associated with fibroid tumors of the uterus. Antifibrinolytic treatment may reduce blood loss perioperatively in myomectomies, and reduce menorrhagia in patients with fibroids. More double randomized studies with larger numbers of participants are necessary to reach more precise and safe conclusions.

Abstract

AIM: To conduct a detailed systematic review of the current evidence on the administration and efficacy of tranexamic acid in patients with menorrhagia due to uterine fibroids.

METHODS: We conducted an electronic search on the following databases PubMed and Medline (1950-2013); (1980-2013); Cochrane library (1993-2013).

RESULTS: A total of 36 articles were retrieved after the initial electronic search. Careful assessment of the retrieved studies led to the final selection of 5 articles for inclusion in the review.

CONCLUSION: Tranexamic acid may reduce blood loss perioperatively in myomectomies. It may reduce the menorrhagia in patients with fibroids, however a stratification of fibroids by size and location is required to define the responses. It is safe in general, with mild adverse effects observed in some cases. More studies with a double-blind randomized design and larger numbers of participants are necessary to reach more precise and safe conclusions.

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INTRODUCTION

Worldwide, approximately 235 million women are affected by uterine fibroids and about 20%-40% of women will be diagnosed with leiomyomas at some point in their life, though only a fraction of those will cause problems or require treatment^[1]. Uterine fibroid tumors or leiomyomas very often lead to abnormal menstrual bleeding or menorrhagia^[2]. Menorrhagia is abnormal extensive menstrual bleeding in cases where the quantity of the overall blood loss exceeds 80 mL in every menses^[3].

The treatment of uterine leiomyoma may be surgical or conservative. Surgical management consists of total

or subtotal hysterectomy and myomectomy, but in some cases less invasive procedures, such as uterine artery embolization are successful^[3].

Data show the presence of extensive fibrinolysis in the menstrual blood of women suffering from menorrhagia, and this has triggered the use of antifibrinolytic drugs as a therapeutic option^[4]. Tranexamic acid (cyklokapron) is a non-hormonal medication that decreases menstrual hemorrhage and it is an excellent therapeutic option in patients with menorrhagia who opt for nonhormonal management^[5]. Tranexamic acid achieves hemostasis and elicits its antifibrinolytic action by reversible block of the locus that connects with lysine on plasminogen molecules. It inactivates the plasminogen activator of the endometrium and thus stops fibrinolysis and degradation of the clotting complexes^[6]. Tranexamic acid has been administered on a daily basis to reduce excessive hemorrhaging and the need for transfusion during and after major cardiac or orthopedic surgeries^[7].

In the international literature, several randomized clinical trials have been published which have evaluated and reviewed the efficacy of tranexamic acid in the management of abnormal gynecological hemorrhagic conditions. It is not certain how efficient tranexamic acid is in treating women with normal reproductive function and diagnosed with abnormal bleeding caused by uterine fibroids^[8].

Aim

The aim of the study was to conduct a systematic review of the current evidence on the administration and efficacy of tranexamic acid in patients with menorrhagia caused by uterine myomas. The administration of tranexamic acid during the preoperative and postoperative period as a method of reducing blood loss is also reviewed. No previous systematic review of the use of tranexamic acid in women with fibroids has been reported.

MATERIALS AND METHODS

Search strategy

We conducted an electronic search on the following databases PubMed and Medline (1950-2014); EMBASE (1980-2013); Cochrane library (1993-2014). The *Medical Subject Headings* which were utilized were as follows: “tranexamic acid” and “fibroids” and “myomas” and “leiomyomas” and “myomectomy”.

Manuscripts written in English or French languages were selected for inclusion in the study. The retrieved studies were scrutinized and their references were examined carefully in order to reveal any relevant studies not identified initially by the electronic search. The included studies were reviewed independently by two authors (PP and AK). In cases of discrepancy and lack of evidence, the corresponding authors of the studies were contacted to provide further information and clarification. Studies from conferences and scientific meetings were also searched.

From each study, we gathered the following clinical data: author and year of publication; country of origin of

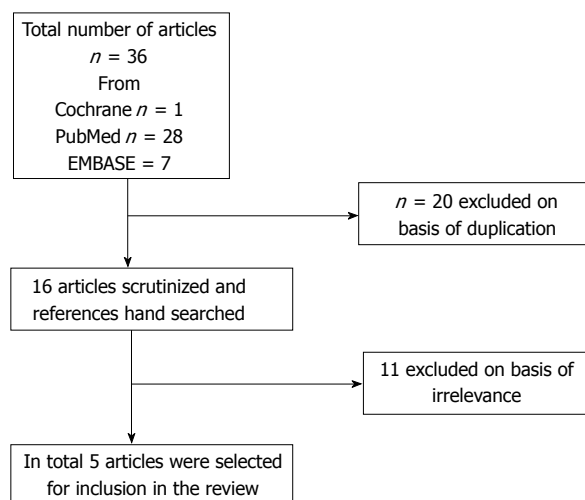


Figure 1 Flowchart of the selection of studies.

study; type of study; number of participants in the study; aim of the study; dosage, type and length of administration of tranexamic acid; data about adverse effects and the conclusion of study results. The clinical data were collected and presented in a table according to chronological order of publication.

Inclusion criteria

All the studies reported the administration of tranexamic acid for treatment of hemorrhage in women of reproductive age with symptomatic fibroids. In addition, studies that reported the administration of tranexamic acid preoperatively or postoperatively to myomectomy procedures were selected for inclusion.

Exclusion criteria

Studies that reported the use of tranexamic acid in pregnant women and in women diagnosed with malignant gynecological disease were excluded. Also studies in women with non-symptomatic fibroids, postmenopausal women and women with hemorrhage related to reasons other than fibroids (dysfunctional uterine bleeding; hematological disorders) were not included in the review.

Quality assessment of studies

The quality assessment of studies was performed according to the guidelines reported from The Scottish Intercollegiate Guidelines Network (SIGN)^[9].

RESULTS

A total of 36 articles were retrieved after the initial electronic search. These 36 articles were scrutinized for duplicate results. The flowchart diagram of the selection process is shown in Figure 1. Eleven articles were excluded because they were not concerned with management of menorrhagia in women with fibroid tumors. Careful assessment of the retrieved studies led to the final selection of 5 articles to be included in the study. A summary of the selected articles is presented in Table 1^[10-14]. The

Table 1 Summary of the selected studies

Ref.	Country	Type of study	Mean age	Symptomatology	Participants	Aim of the study	Regimen administration	Results	Adverse effects	Comment
Lakhani <i>et al</i> ^[10]	United Kingdom	Longitudinal Prospective	42.8	Menorrhagia Pelvic pain	<i>n</i> = 12	Ultrasound assesment of PI and RI of UA in women with TA administration	Tranexamic acid P.O. 1 g x 3 for 2 cycles	No significant changes in blood loss or PI and RI	Not reported	No changes in UA-PI resistance in women with fibroids
Caglar <i>et al</i> ^[11]	Turkey	Prospective randomized double-blind placebo	34.2	Menorrhagia Pelvic pain	<i>n</i> = 50 (TA) <i>n</i> = 50 (Placebo)	To compare the perioperative blood loss in patients undergoing myomectomy and taking TA with patients not taking TA	Tranexamic acid 10 mg/kg <i>iv</i> (max 1 g) 15 min before incision	Significant statistical differences in two groups postoperative, total blood loss and duration of surgery (<i>P</i> < 0.01) in favor of TA	Not reported	TA does not reduce perioperative blood loss nor Hb levels. It reduces postoperative and total blood loss and surgery time in correlation with myoma size. However further investigations required
Ip <i>et al</i> ^[12]	Hong Kong	Observational	43.8 ± 25	Menorrhagia Pelvic pain	<i>n</i> = 22	Pathology assesment of fibroid specimen in women receiving TA	Tranexamic acid Per os dosage not reported	Necrosis and infarcts in resected fibroids. Larger diameter fibroids more prone to necrosis changes. Size is an independent factor	Not reported	Authors emphasize the necrosis and thrombosis in fibroids but suggest precaution for complications
Lukes <i>et al</i> ^[14]	United States	Randomized double-blind placebo	36.5	Menorrhagia Pelvic pain	<i>n</i> = 42 (TA) <i>n</i> = 26 (Placebo)	To assess the efficacy and safety of TA for heavy menstrual bleeding	Tranexamic acid Per os 1.3 g daily for 5 d up to 6 cycles	Reduction in menstrual blood loss in women receiving TA compared to placebo. No statistically significant changes in blood loss in patients with fibroids	Mild adverse effects Menstrual cramps Gastrointestinal allergies	TA was effective in the treatment of heavy menstrual bleeding regardless of the presence or absence of fibroids
Eder <i>et al</i> ^[13]	United States	Randomized double-blind placebo	38	Menorrhagia Pelvic pain	<i>n</i> = 96 (TA) <i>n</i> = 51 (Placebo)	To compare the menstrual blood loss in women with fibroids and TA and women with fibroids not taking TA. consisting placebo group	Tranexamic acid Per os 3.9 g/d for 5 d up to 6 menstrual cycles	Menstrual blood loss reduced in women receiving TA (<i>P</i> < 0.001)	3 patients in TA group and 3 in placebo group reported headache	TA was well tolerated and reduced menstrual blood loss

TA: Tranexamic acid; UA: Umbilical artery; UA-PI: Umbilical artery pulsatility index.

reviewed studies originated from Europe^[10,11], Asia^[12] and America^[13,14]. All articles were written in English. The studies were published between 1998 and 2013. The total study population from the 5 studies was 349 women; 206 patients were treated with tranexamic acid and 101 patients were allocated to the placebo groups. All patients were premenopausal with a mean age of 37.65 ± 3.2 years. All patients in the study groups presented with menorrhagia and pelvic pain due to uterine fibroids. Three studies had a double-blind randomized design^[11,13,14], one had an observational design^[12] and one had a prospective longitudinal design^[10]. Tranexamic acid was administered orally in four studies^[10,12-14], and intravenously in one study^[11]. Tranexamic acid reduced the blood loss perioperatively in women undergoing myomectomy in comparison to women not receiving tranexamic acid according to the authors^[11]. Management of excessive bleeding during the menstrual cycle with tranexamic acid decreased hemorrhage despite the existence of myomas^[13].

Furthermore, tranexamic acid reduced the quantity of bleeding in patients with menorrhagia in a pivotal phase III randomized double blind study^[14]. The authors reported that they estimated the quantity of blood *via* a validated alkaline hematin method in patients with sonographically confirmed fibroids^[14]. Tranexamic acid did not alter the pulsatility index during ultrasound assessment of women with fibroids^[10]. Significant pathologic changes were noted in specimens from women who received tranexamic acid and underwent myomectomy. Very mild complications of treatment were seen in 2 studies^[13,14], and 3 women in a single study reported headache^[14].

Quality of the studies

The quality assessment of the selected studies according to SIGN criteria is shown in Table 2. Three studies were graded 2++ (high quality studies)^[11,13,14] and 2 studies were graded 2+ (well conducted studies)^[10,12]. All studies were conducted in University teaching hospitals^[10-14], 3 were conducted in a single setting^[10-12], and 2 were conducted in multicenter settings^[13,14]. Three studies received financial support from pharmaceutical companies^[10,13,14].

DISCUSSION

In practice, tranexamic acid has been administered in many clinical situations in which the inhibition of fibrinolysis has shown beneficial effects in managing hemorrhage. The use of tranexamic acid in Obstetrics and Gynecology as a conservative method for reducing blood loss has been extensive^[15]. TA provides a non-hormonal, treatment for patients with excessive hemorrhage during the menstrual period^[13]. How tranexamic acid manages menorrhagia provoked by leiomyoma is still unclear and unknown due to the limited data.

In the current review, according to the reported studies, tranexamic acid is a safe treatment and may reduce menorrhagia in women with fibroids. It reduces the blood loss perioperatively with no adverse effects in women un-

dergoing laparotomy and myomectomy. Tranexamic acid causes necrosis in myomas but does not alter pulsatility indices in ultrasound assessment. However, the current review has some limitations because of the quantity and quality of studies published in the literature and the presence of bias related to the size and location of fibroids.

Despite the fact that tranexamic acid administration has shown a risk for complications like thrombosis and embolism due to its antifibrinolytic effect, thromboembolic events were not been reported in the selected studies. Only mild headaches, allergies and discomfort were reported in a small population of patients^[13,14]. In the study by Lukes *et al.*^[14], the authors did not specify the exact type and number of adverse effects in patients with fibroids, and stated that the most common adverse effect was menstrual discomfort.

Tranexamic acid has been administered widely in Scandinavian and European countries in general as a first-line management option for menorrhagia since the 1970s, and data have shown no increase in the frequency of adverse clotting disorders^[16,17]. However, the optimal dose and duration of treatment with tranexamic acid has not been established^[18].

The efficacy and safety of tranexamic acid when given intravenously for peri- and postoperative hemorrhage has been investigated more in orthopedic and cardiovascular surgical interventions^[19]. In the study by Caglar *et al.*^[11], the authors reported that tranexamic acid succeeded in decreasing perioperative blood loss during excision of myomas; however, they emphasized the importance of various parameters such as type of surgery, surgical skills, and duration of surgery for perioperative blood loss. In the same study, the location and type of myoma (subserous, intramural, submucous) were highlighted and also the number and size of fibroid tumors. Multiple fibroid tumors may increase the duration of surgery in contrast to a single large myoma > 6 cm^[12].

Ip *et al.*^[12] concluded that tranexamic acid induced necrosis of fibroids. Larger fibroids were more prone to necrosis. The authors emphasized the significance of tranexamic acid in conservative management of fibroids, thus sparing unnecessary surgical interventions. However possible complications such as pelvic pain and low grade fever maybe present in these patients^[12].

It has been reported in clinical studies that the levels of plasminogen activator are elevated 30 min after the initiation of surgery, and this mechanism may elicit a reduction in bleeding in surgical patients^[11,19].

One randomized study investigated whether tranexamic acid was effective in comparison with placebo for the management of menorrhagia in patients with no pathological findings in the pelvis^[13]. The factual limitations of this study were that in women diagnosed with fibroid tumors, myomas were not found in large numbers and their size was not significant to justify surgical removal. Although the goal of this trial was not to assess the effect of tranexamic acid on abnormal vaginal bleeding caused by myomas, outcomes showed that tranexamic acid was effective in treating heavy menorrhagia, and this was not related

Table 2 Quality assessment of the studies according to Scottish Intercollegiate Guidelines Network guidelines

Ref.	Setting	Sign grade	Interpretation
Lakhani <i>et al</i> ^[10]	University teaching hospital	2+	Well conducted study
Caglar <i>et al</i> ^[11]	University teaching hospital	2++	High quality study
Ip <i>et al</i> ^[12]	University teaching hospital	2+	Well conducted study
Lukes <i>et al</i> ^[14]	University teaching hospital	2++	High quality study
Eder <i>et al</i> ^[13]	Private research institution and University teaching hospital	2++	High quality study

to the presence of absence of myomas. However, based on the design of the study, it is hard to postulate that treatment with tranexamic acid is influenced by the size and type of the fibroids^[18]. In the study by Lakhani *et al*^[10], women with fibroids were found to have no significant changes in various sonographic parameters. However, these findings may exhibit bias and limitations because the women were not divided into different groups with different sizes and types of myomas^[18].

The Food and Drug Administration approved tranexamic acid 650 mg (Lysteda-Ferring) in November 2009. Treatment with tranexamic acid while using hormonal contraceptives may increase the risk of developing thrombosis, cardiac complications, and stroke^[20].

Tranexamic acid has been used extensively in patients with heavy menstrual bleeding with good results, and enough evidence is available to support its use. Tranexamic acid may reduce blood loss perioperatively in myomectomies, and may reduce menorrhagia in patients with fibroids, but stratification of fibroids by size and location is required to define the responses to tranexamic acid. Physicians should be aware that tranexamic acid may cause drug-induced necrosis of fibroids and surgical management can be avoided, but complications such as pelvic pain and low grade fever can be present in these patients. It is safe in general, and mild adverse effects are observed in some cases. More studies of a double-blind randomized design and larger numbers of participants are required to reach clearer conclusions about the use of tranexamic acid in patients with fibroids.

COMMENTS

Background

Menorrhagia due to fibroid tumors of the uterus is one the leading causes of abnormal menstrual bleeding. Tranexamic acid is non-hormonal and has been used previously for the treatment of dysfunctional uterine bleeding. The role of tranexamic acid in the treatment of abnormal menstrual bleeding due to uterine fibroid tumors is unclear. The authors have reviewed the literature and demonstrated that tranexamic acid may reduce bleeding in myomectomies and also may reduce the amount of bleeding in patients with menorrhagia caused by uterine fibroids.

Research frontiers

Tranexamic acid may reduce blood loss in patients with menorrhagia due to

fibroids. It may cause drug-induced necrosis of fibroids and surgical management can be avoided, but complications such as pelvic pain and low grade fever can be present in these patients.

Innovations and breakthroughs

The study showed that tranexamic acid reduced blood loss in patients undergoing myomectomy. It may cause necrosis in fibroids and may reduce the menorrhagia due to fibroids. Tranexamic acid has shown mild adverse effects during its administration. It may be used in patients who do not want hormonal treatment.

Applications

To ensure that tranexamic acid can be used in patients with menorrhagia caused by uterine fibroids, further double-blind randomized studies are required in order to ensure that the regimen is safe, efficient and does not cause severe effects.

Terminology

Tranexamic acid is a hemostatic agent that elicits its antifibrinolytic action by reversibly blocking the lysine-binding sites on plasminogen molecules. It inactivates the plasminogen activator in endometrial cells and thus stops fibrinolysis and degradation of the clotting complexes. A number of studies have reported the use of tranexamic in reducing blood loss in cardiac and orthopedic operations. Tranexamic acid has been used to decrease blood loss in patients with menorrhagia. Menorrhagia is abnormal extensive menstrual bleeding where the quantity of overall blood loss exceeds 80 mL in every menses.

Peer review

The authors here performed a systematic review of the current evidence on the administration and efficacy of Tranexamic acid for these patients.

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Reconstruction using a pedicled upper arm fillet flap after excision of a malignant peripheral nerve sheath tumor: A case report

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Key words: Reconstruction; Flaps; Neurofibromatosis; Malignant Peripheral Nerve Sheath Tumor; Sarcoma

Core tip: Here we present a rare case on the use of a pedicled fillet flap of the upper arm for chest wall reconstruction after excision of a malignant peripheral nerve sheath tumor in a patient with neurofibromatosis. This case report describes a reconstructive procedure that is rarely described in the literature as a viable option for soft tissue coverage of shoulder and chest wall defects after an oncologic resection.

Singla P, Kachare SD, Fitzgerald TL, Zeri RS, Haque E. Reconstruction using a pedicled upper arm fillet flap after excision of a malignant peripheral nerve sheath tumor: A case report. *World J Clin Cases* 2014; 2(12): 899-902 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i12/899.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i12.899>

Abstract

Non-salvageable extremities have been utilized for harvesting fillet flaps as part of the "spare parts" concept in traumatic and oncologic settings. Here we report on the use of a pedicled fillet flap of the upper arm for chest wall reconstruction after excision of a malignant peripheral nerve sheath tumor in a patient with neurofibromatosis. Pedicled flaps as part of the "spare parts" concept provide the advantage of reduced donor-site morbidity, immediate closure, intact vasculature, and adequate soft tissue coverage of large defects. Malignant peripheral nerve sheath tumor is a rare aggressive tumor with a poor prognosis that may result in large defects post resection. Limited data describes the use of pedicled fillet flaps of the upper extremity. We report the use of a pedicled fillet flap of the upper arm as a viable option that can be successfully used for coverage of soft tissue defects of the shoulder and chest wall post complex resections in an oncologic setting.

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INTRODUCTION

The use of non-salvageable extremities for harvesting fillet flaps has been utilized for reconstruction as part of the "spare parts" concept in traumatic and oncologic settings. Fillet flaps have been extensively characterized based on their clinical value and can be used as pedicled or free flaps^[1], however there is limited data describing the use of fillet flaps of the upper extremity^[2]. Here we report a rare case of harvesting a pedicled fillet flap of the upper arm for chest wall reconstruction after excision of a malignant peripheral nerve sheath tumor (MPNST) in a patient with neurofibromatosis.

CASE REPORT

A 42-year-old female with a history of neurofibromatosis presented to plastic surgery clinic with complaints

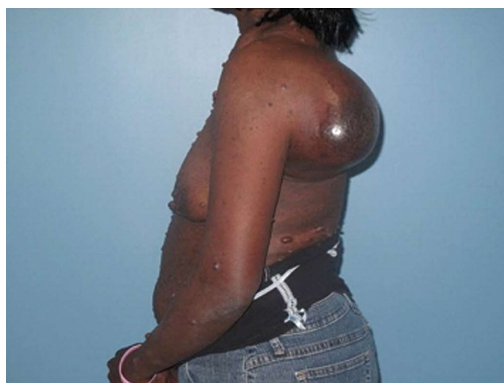


Figure 1 Left shoulder mass at initial presentation.

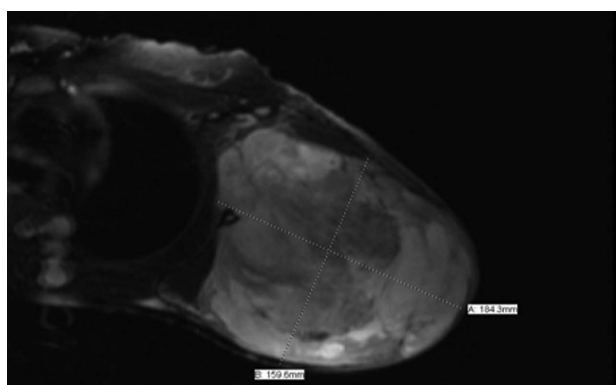


Figure 2 Magnetic resonance imaging of left shoulder, Mass: 18.4 cm × 15.9 cm × 20 cm.

of a left shoulder mass growing for the past two years that caused significant pain with movement (Figure 1). Magnetic resonance imaging (MRI) revealed 18.4 cm × 15.9 cm × 20 cm mass concerning for possible malignant degeneration of a neurofibroma based on size and clinical history (Figure 2). Neoadjuvant chemoradiation per National Comprehensive Cancer Network guidelines for resectable soft tissue sarcomas with potential for adverse functional outcomes^[3] was discussed. Patient was lost to follow up after initial planning and when she returned, she was unable to lay supine secondary to pain from the tumor, which had obvious necrosis with bleeding. Chest computerized tomography (CT) revealed a left large axillary mass with scapular erosion as well as small pulmonary nodules suggestive but not diagnostic of metastatic disease. A decision was made to abandon neoadjuvant therapy and surgically resect the axillary mass followed by adjuvant therapy with close observation of pulmonary lesions.

After the scapula was disarticulated and the tumor excised, it became clear that the arm would be of limited functional use and a modified forequarter amputation with vascular preservation of the upper extremity was performed. A regional flap could not be done due to the wide defect and need for post-operative radiotherapy. Use of a pedicled latissimus dorsi was contraindicated due to tumor invasion, therefore in order to provide immediate closure, without the added time and risk of performing

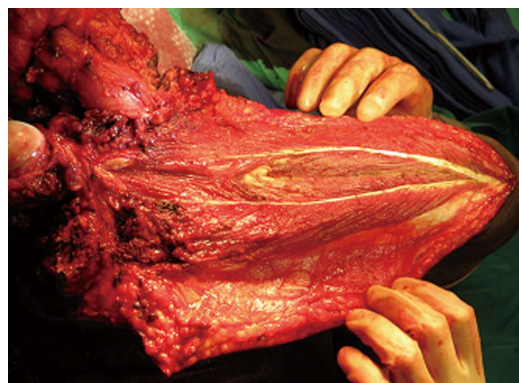


Figure 3 Fillet flap of the upper arm post subperiosteal dissection and removal of the humerus.

a free flap, a musculocutaneous pedicled fillet flap of the left upper arm was chosen for reconstruction.

An incision was made on the posterior aspect of the left upper extremity starting at the shoulder joint and extending below the elbow. Skin and triceps muscles were dissected in the midline and then a subperiosteal dissection was performed (Figure 3). Next, the humerus was removed and the arm transected just below the elbow after identifying and ligating the neural and vascular structures. The flap was rotated into the defect without any tension (Figure 4).

Post-operative pathology report demonstrated a T2bN0 grade 2 or stage II b^[3] MPNST. The tumor was 24 cm × 24 cm × 19 cm in size with focally positive margins. After meeting with radiation and medical oncology, the patient agreed to undergo adjuvant chemoradiation. The patient was initiated on a chemotherapy regimen consisting of doxorubicin and ifosfamide and scheduled for radiation therapy. However, three months after surgery, patient had local recurrence of a mass at her left shoulder. Patient was referred to an outside facility closer to home for adjuvant therapy and this was delayed secondary to patient compliance. CT of the chest and shoulder revealed large recurrent solid and cystic mass in the left shoulder region and left upper anterolateral chest wall. The patient died 5 mo postoperatively from hemorrhagic conversion of metastatic lesions in the brain.

DISCUSSION

The use of fillet flaps from non-salvageable or amputated extremities has been successfully used for reconstruction as part of the “spare parts” concept^[1]. Pedicled musculocutaneous flaps are commonly used for reconstruction; however, the use of a pedicled fillet flap of the upper arm has not been well reported in the literature^[2].

Forequarter amputations may be necessary for locally aggressive bone and soft tissue tumors invading the axilla, shoulder or scapula^[4]. However, multiple variables, such as size and location of the defect, exposure of nerves, tendons, vessels and bones, as well as need for post-operative radiation, must be taken into consideration when deciding on type of reconstruction. Skin grafts do not

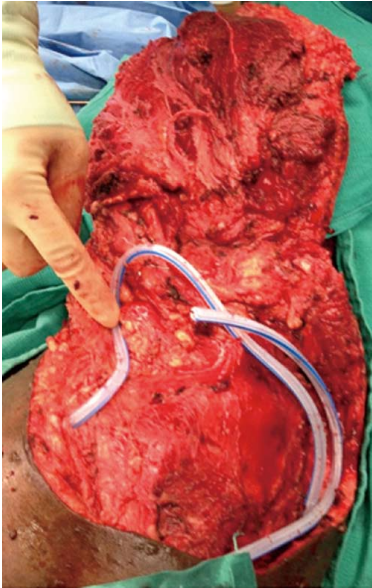


Figure 4 Fillet flap with drains, prior to closure.

provide adequate coverage for large, deep wounds, and are not durable in patients needing post-operative radiation. Therefore, flap coverage with either a pedicled or free flaps are the appropriate choice to provide adequate, immediate coverage post-resection in order to initiate adjuvant radiation therapy^[5]. In patients undergoing an amputation, the use of fillet flaps as part of the “spare parts” concept provides these similar advantages with the added benefit of reduced donor-site morbidity^[1,5].

Previous studies describe the use of free forearm fillet flaps in patients who underwent an upper extremity amputation for cancer, however the use of an upper arm pedicled fillet flap has not been well reported^[2,6]. The use of an upper arm flap may have been contraindicated due to local invasion of the tumor^[2]. However, in our patient, the lack of tumor invasion into the upper arm or into the vascular inflow in the axilla allowed the opportunity to perform a pedicled fillet flap. Not only did the upper extremity flap allow for adequate, tension free coverage, it reduced the risks associated with a free flap. Chao and colleagues demonstrated that patients who received adjuvant radiotherapy after free fillet flaps were significantly more likely to have graft loss as compared to those who received neo-adjuvant radiation^[7]. Since our patient was not appropriate for neo-adjuvant radiation, performing an upper-extremity pedicled fillet flap allowed us to reduce the risk of post-operative wound complications associated with adjuvant radiation therapy. Pedicled latissimus dorsi flaps for reconstruction after forequarter amputations have also been described^[8], but due to tumor invasion in our patient as well as added donor site morbidity, an upper arm fillet flap was decided to be the most appropriate choice for reconstruction.

MPNST is a rare primary chest wall tumor with an incidence of 0.001% in the general population. It commonly presents as an enlarging painful mass and arises from Schwann cells or neural crest cells in a peripheral

nerve or its sheath. Patients with NF-1 are at increased risk of developing MPNST through malignant degeneration of plexiform neurofibromas^[9]. MPNSTs are considered highly malignant, associated with a poor prognosis, have a high risk of local recurrence, and are associated with distant metastasis, most commonly to the lungs^[9,10]. MRI remains the gold standard for diagnosis after which the treatment of choice is surgical resection^[9]. Definite wide excision, negative surgical margins, with neo-adjuvant or adjuvant radiotherapy is currently recommended for treatment of resectable tumors in patients with MPNSTs^[3,9]. Poor prognostic indicators include tumor size greater than 5 cm, local recurrence, high tumor grade, positive surgical margins, association with neurofibromatosis type I (NF-1), and truncal location^[9]. Patients with NF-1 should be educated about the increased risk for developing MPNST and be advised to contact their physician should rapidly enlarging masses, pain, or neurologic changes occur. Given the poor prognosis for MPNST, early initiation of treatment provides the best chance for survival^[9].

Overall, this case represents a rare description of the use of a pedicled musculocutaneous flap from the upper arm for reconstruction after resection of MPNST. Given that MPNST is an aggressive tumor that may present with large defects post resection, the use of a pedicled fillet flap of the upper arm is a viable option that can be successfully used for coverage of soft tissue defects of the shoulder and chest wall^[9]. The advantage of immediate wound closure, avoidance of donor-site morbidity, and reduced operative time over a free flap makes this procedure a reliable method for complex reconstructions in an oncologic setting^[1].

COMMENTS

Case characteristics

A 42-year-old female with a history of neurofibromatosis presented to plastic surgery clinic with complaints of a left shoulder mass growing for the past two years that caused significant pain with movement.

Clinical diagnosis

Patient has a tense, large, protruding mass over her left scapula that is excruciatingly tender with intact flexor and extensor function of the hand and elbow and gross sensation but limited shoulder function secondary to significant pain.

Differential diagnosis

Malignant peripheral nerve sheath tumor, cellular schwannoma, fibrosarcoma, synovial sarcoma.

Laboratory diagnosis

White blood cell: 9.80 k/ μ L; hemoglobin: 9.9 g/dL.

Imaging diagnosis

Magnetic resonance imaging revealed 18.4 cm \times 15.9 cm \times 20 cm mass concerning for possible malignant degeneration of a neurofibroma based on size and clinical history and chest computerized tomography revealed a left large axillary mass with scapular erosion.

Pathological diagnosis

Post-operative pathology report demonstrated a T2bN0 grade 2 or stage IIb malignant peripheral nerve sheath tumor and the tumor was 24 cm \times 24 cm \times 19 cm in size with focally positive margins.

Treatment

Surgical resection of the mass using a pedicled upper arm fillet flap was performed and post operatively, the patient was initiated on a chemotherapy regimen consisting of doxorubicin and ifosfamide and scheduled for radiation

therapy.

Related reports

Pedicled musculocutaneous flaps are commonly used for reconstruction; however, the use of a pedicled fillet flap of the upper arm has not been well reported in the literature.

Term explanation

A pedicled flap contains tissue that remains attached to the original donor site with intact vasculature and is transposed to a new location which is in contrast to a free flap where tissue is detached from its original donor site and transferred to another location.

Experiences and lessons

The advantage of immediate wound closure, avoidance of donor-site morbidity, and reduced operative time over a free flap makes the use of a pedicled fillet flap of the upper arm a viable option that can be successfully used for coverage of soft tissue defects of the shoulder and chest wall for complex reconstructions in an oncologic setting.

Peer review

This case report describes a novel reconstructive procedure that can be used to cover the amputated upper arm with a pedicled fillet flap.

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Cecal bascule herniation into the lesser sac

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Author contributions: Jacobs MJ designed and reviewed the study; Makarawo T and Macedo FI collected the data, and wrote the paper.

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Key words: Cecal; Bascule; Hernia; Internal; Foramen; Winslow

Core tip: Cecal bascule is a rare and overlooked cause of large bowel obstruction in which a mobile cecum folds anteriorly and superiorly leading to obstruction of ascending colon. Although cecal bascule has been described in association with mechanical bowel obstruction in the literature, its association with this type of internal hernia has never been described before. The management of internal herniation of a cecal bascule is always surgical even in the absence of peritonitis, either cecopexy or right hemicolectomy depending on the viability of the bowel segment involved.

Abstract

Cecal bascule is a rare cause of bowel obstruction in which a mobile cecum folds anteriorly and superiorly over the ascending colon. Herein, we present the first case of internal herniation of a cecal bascule into the lesser sac through the foramen of Winslow, aiming at discussing radiological findings, differential diagnosis, and surgical management of this uncommon condition. A 75-year-old female presented to the emergency room with an 18-h history of sudden onset sharp, progressively worsening abdominal pain associated with vomiting. Physical exam revealed abdominal distention and epigastric tenderness while initial laboratory tests were unremarkable. Computed tomography of her abdomen and pelvis showed a loop of distended colon within lesser sac without signs of bowel ischemia or perforation. On exploratory laparotomy, a cecal bascule was found herniating into lesser sac *via* foramen of Winslow. Upon reduction, the cecum appeared viable therefore a cecopexy was performed without bowel resection. Unlike cecal volvulus, cecal bascule consists of no axial rotation of the bowel with no mesenteric vascular compromise and therefore ischemia would only occur from intraluminal tension or extraluminal compression from the borders of foramen of Winslow. The management of internal herniation of a cecal bascule is always surgical including anatomic resection or cecopexy.

Makarawo T, Macedo FI, Jacobs MJ. Cecal bascule herniation into the lesser sac. *World J Clin Cases* 2014; 2(12): 903-906 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i12/903.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i12.903>

INTRODUCTION

Cecal bascule is an extremely rare condition in which the bowel folds anteriorly and superiorly over the ascending colon^[1]. Its similarity with cecal volvulus has led to misdiagnosis, although the absence of axial rotation of the bowel in cecal bascule is an importance difference that influences presentation. In cecal bascule, patients usually present with less critical illness than those with cecal volvulus as there is no torsion of the mesenteric vasculature^[2]. Symptoms are therefore mostly related to bowel obstruction, particularly in the presence of a functional ileocecal valve causing a closed-loop obstruction. Diagnosis is often challenging because of equivocal image findings in addition to its rare occurrence. We, herein, present a rare case of cecal bascule herniating into the lesser sac in a patient with obstructive signs, and discuss the diagnosis, and operative management of this rare

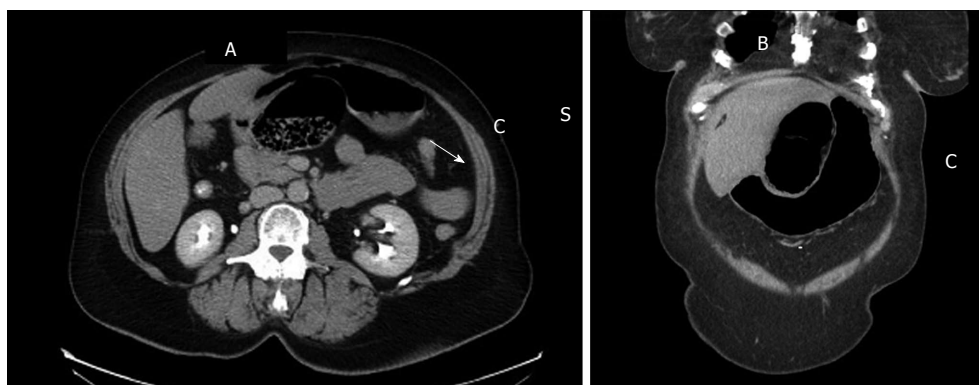


Figure 1 Computed tomography of abdomen and pelvis showing a loop of distended colon within lesser sac without bowel ischemia or perforation. Radiological features include: (1) the cecum herniated (C) into the lesser sac behind the stomach (S) (A and B); (2) the presence of mesentery (white arrow) between the portal vein and inferior vena cava (A); and (3) the presence of gas or fluid in the lesser sac with its 'beak' directed toward the foramen of Winslow (B).

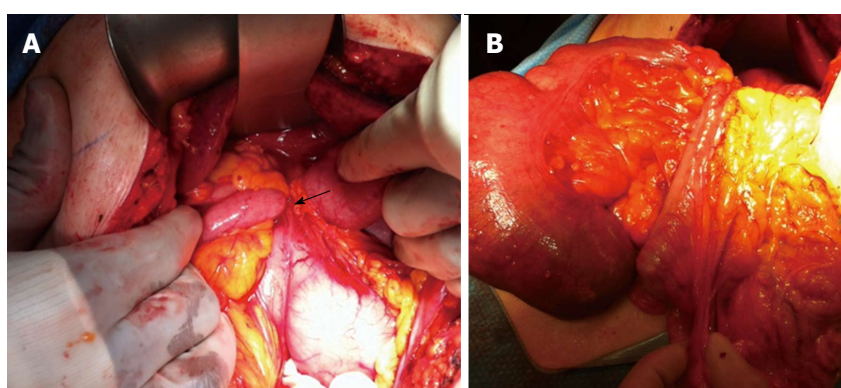


Figure 2 Intraoperatively, a cecal bascule was found herniating into lesser sac via foramen of Winslow (A). Upon reduction, the cecum appeared viable (B).

condition.

CASE REPORT

A 75-year-old female presented to the emergency room with an 18-h history of sudden onset, progressively worsening abdominal pain associated with vomiting. The patient had a previous history of hiatal hernia with gastroesophageal reflux symptoms and previous hysterectomy and tonsillectomy. The patient presented with abdominal distention and epigastric tenderness without diffuse peritoneal signs. Laboratory tests were unremarkable, with no leukocytosis but computed tomography (CT) of the abdomen and pelvis showed a loop of distended colon within lesser sac with no signs of bowel ischemia or perforation (Figure 1).

The patient underwent exploratory laparotomy during which the cecum was noted to be folded anteriorly over the ascending colon and herniating into lesser sac *via* the foramen of Winslow (Figure 2A) resulting in colonic obstruction. To reduce the internal hernia, the lesser omentum was incised revealing the absence of underlying adhesive bands tethering the cecum in place, hence allowing the gentle withdrawal of the bowel back through foramen of Winslow. Upon reduction, the cecum and terminal ileum appeared viable (Figure 2B). Therefore,

lysis of a single adhesive band to the terminal ileum was performed prior to cecopexy with fixation of the cecum inferolaterally to the right lateral abdominal wall, negating the need for bowel resection.

The patient made an uneventful post-operative recovery, tolerating full oral intake within 48 h of the surgery and being discharged on post-operative day four. The patient has had no further episodes of bowel obstruction, remaining symptom-free after almost a year of follow-up.

DISCUSSION

Cecal bascule is a rare and overlooked cause of large bowel obstruction in which a mobile cecum folds anteriorly and superiorly leading to obstruction of ascending colon. It has been estimated that approximately 10% to 15% of labeled "cecal volvulus" episodes were actually cecal bascules^[3]. The formation of a cecal bascule is secondary to hypermobility of the cecum and intestinal distention^[4,5]. This excessive mobility can occur as a result of defective retroperitoneal fixation of the cecum due to incomplete intestinal rotation during embryogenesis, or the persistence of ascending mesocolon. It may also occur postoperatively after dissection of peritoneal attachments^[6]. The formation of adhesions may also play a role by creating a point of fixation, allowing the formation of

the bascule.

The clinical presentation of cecal bascule is usually similar to postoperative ileus including nausea, vomiting and abdominal distention and pain. However, unlike in cecal volvulus, the patients are not as critically ill because there is no axial torsion of the mesenteric vasculature, which leads to bowel ischemia. However, it must be noted that delay in diagnosis with unrelieved bowel obstruction causing increased intraluminal tension or extraluminal compression from an internal hernia would ultimately result in bowel ischemia and eventual perforation. Diagnosis is often challenging due to its rare nature. Plain abdominal X-rays may show a dilated cecum in the right upper quadrant with or without small bowel obstruction. CT scan of the abdomen, which is more helpful, may demonstrate a dilated cecum anterior to the ascending colon and an ileocecal valve located in the right upper quadrant^[4]. In our case, the CT scan did show an internal hernia containing a loop of distended colon within lesser sac, although it was unclear whether it was transverse colon or ascending colon that had herniated through the foramen of Winslow.

Herniation of abdominal viscera through the foramen of Winslow into the lesser sac occurs rarely, accounting for 8% of all internal hernias^[7]. Hernias through this foramen have been described as containing small bowel, right colon, and rarely, gallbladder or transverse colon. Potential predisposing factors include hypermobile mesentery, enlargement of the foramen of Winslow, or absence of fusion of the ascending colon to the posterior abdominal wall^[7,8]. Although cecal bascule has been described in association with mechanical bowel obstruction in literature, its association with this type of internal hernia has never been described before^[8-14]. Indeed, it is possible that the term 'bascule' which is derived from a French word meaning "a bridge with a movable section hinged about a horizontal axis", is not widely applied, and therefore underdiagnosed as a cause for cecal herniation.

The management of bowel obstruction secondary to cecal bascule should be surgical. In this case, simple reduction and cecopexy was technically feasible, safe, and had a satisfactory outcome. Although right hemicolectomy has also been advocated even with the cecum being viable to prevent recurrence^[9], there are no reported cases of recurrence of cecal bascule following cecopexy only^[10-12]. Therefore, we would recommend that segmental resection be reserved for cases with associated ischemia or perforation. Closure of the foramen has also not been advocated due to increased risk of portal vein thrombosis or hepatic artery and/or bile ducts injury^[13,14].

In conclusion, we presented a rare case of an internal hernia containing a cecal bascule into the lesser sac through the foramen of Winslow. Despite its rare occurrence, cecal bascule should be in the differential diagnosis armamentarium of bowel obstruction, especially in patients with a markedly distended cecum in the absence of peritoneal signs. The management of internal herniation of a cecal bascule is always surgical even in the absence

of peritonitis, and includes either cecopexy if the cecum is viable or right hemicolectomy if it appears ischemic or non-viable.

COMMENTS

Case characteristics

A 75-year-old female presented to the emergency room with an 18-h history of sudden onset, progressively worsening abdominal pain associated with vomiting.

Clinical diagnosis

On physical examination, she had abdominal distention and epigastric tenderness without diffuse peritoneal signs.

Differential diagnosis

Mechanical obstruction due to adhesions, ileus, cecal volvulus, cecal bascule.

Imaging diagnosis

Computed tomography of abdomen and pelvis showing a loop of distended colon within lesser sac without bowel ischemia or perforation.

Treatment

The patient underwent exploratory laparotomy, reduction of cecal herniation into the lesser omentum and cecopexy.

Term explanation

Cecal bascule occurs when a mobile cecum folds anteriorly and superiorly leading to obstruction of ascending colon. The term "bascule" is derived from a French word meaning "a bridge with a movable section hinged about a horizontal axis", is not widely applied, and therefore may be underdiagnosed.

Experience and lessons

This is the first case in the literature describing a hernia into the lesser sac from a cecal bascule. The management of bowel obstruction secondary to cecal bascule should be surgical.

Peer review

This manuscript highlights the clinical presentation of a rare cause of large bowel obstruction and provides insights into the management of cecal bascule.

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Tentorial dural arteriovenous fistula presenting as myelopathy: Case series and review of literature

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Abstract

Dural arteriovenous fistula (DAVF) is a rare type of cerebral arteriovenous malformation. Common presenting symptoms are related to hemorrhage. However, rarely these patients may present with myelopathy. We present two cases of DAVF presenting as rapidly progressive myelopathy. Two treatment options are available: microsurgical interruption of the fistula and endovascular embolization. These treatment options of DAVFs have improved significantly in the last decade. The optimal treatment of DAVFs remains controversial, and there is an ongoing debate as to whether primary endovascular or primary microsurgical treatment is the optimal management for these lesions. However, despite treatment a high percentage of patients are still left with severe

disability. The potential for functional ambulation in patients with DAVF is related to the time of intervention. This emphasizes the importance of early diagnosis and early intervention in DAVF. The eventual outcome may depend on several factors, such as the duration of symptoms, the degree of disability before treatment, and the success of the initial procedure to close the fistula. The usage of magnetic resonance imaging and selective angiography has significantly improved the ability to characterize DAVFs, however, these lesions remain inefficiently diagnosed. If intervention is delayed even prolonged time in rehabilitation does not change the grave prognosis. This review outlines the presentation, classification and management of DAVF as well as discussing patient outcomes.

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Key words: Dural arteriovenous fistula; Myelopathy; Vascular malformation; Cognard classification; Microsurgery; Onyx embolization

Core tip: Tentorial dural arteriovenous fistulas (DAVF) are an uncommon entity, and myelopathy as a result of these AV fistulas is even more uncommon. We present two cases of myelopathy as a result of dural AV fistulas. This review highlights the classification of dural AV fistulas, the various diagnostic modalities available for diagnosis and management strategies employed for the treatment of DAVF. We also stress the importance of a timely diagnosis and its impact on patient outcomes and recovery.

Gross R, Ali R, Kole M, Dorbeistein C, Jayaraman MV, Khan M. Tentorial dural arteriovenous fistula presenting as myelopathy: Case series and review of literature. *World J Clin Cases* 2014; 2(12): 907-911 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i12/907.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i12.907>

INTRODUCTION

Dural arteriovenous fistulas (DAVF) constitute 10% to 15% of all intracranial vascular malformations^[1,2]. Tentorial DAVFs account for 8.4% of intracranial DAVFs^[3]. Cranial dural arteriovenous fistulas may give rise to myelopathy due to spinal perimedullary venous drainage causing intramedullary venous hypertension^[4]. However, this is very uncommon, with only 38 cases reported in the literature^[5]. We describe two cases of tentorial dural AV fistulas causing significant myelopathy.

CASE REPORT

Case 1

A 69-year-old male presented to our institution with 3 d of progressively worsening bilateral lower extremity weakness and urinary retention. These symptoms had been preceded by bilateral hand and forearm pain that lasted for 2 d. The patient was presumptively diagnosed with Guillian-Barre Syndrome and treatment started. Magnetic resonance imaging of the brain and of the cervical, thoracic, and lumbar spine showed increased T2/STIR signal intensity in the pons, medulla, and upper cervical spine and multiple small flow voids in the dorsal cervicothoracic spine suggestive of dural arteriovenous malformation (Figure 1). Neurological exam revealed significant weakness in bilateral lower extremities, most pronounced in bilateral hamstrings and in the left tibialis anterior. The upper extremity strength testing was normal. There were no sensory abnormalities. Deep tendon reflexes were increased in the lower extremities. This presentation was thought to be consistent with myelopathy from venous congestion related to a DAVF. He underwent angiography, which confirmed a Cognard V tentorial dAVF fed by the left middle meningeal, tentorial branch of the left ICA, and dural branches of the occipital and posterior auricular arteries, with drainage into cervical spinal veins (Figure 2). The AVF was successfully embolized with Onyx (Covidien Inc., Mansfield, MA) without any residual filling seen following embolization (Figure 3). The patient was discharged to rehab on hospital day 5. He was seen in follow-up 10 wk later, at which point he was noted to be walking independently, without urinary symptoms and only mild proximal lower extremity weakness bilaterally with manual muscle testing grade of 4 out of 5 in bilateral iliopsoas and hamstrings. The Aminoff-logue scale changed from 6 preintervention, to 1 after embolization of dural AV fistula.

Case 2

A 34-year old woman presented with progressively worsening bilateral upper and lower extremity weakness over 1 wk. Past medical history was significant for temporal pilocytic astrocytoma resection with subsequent whole brain radiation at age 12. Examination revealed quadriplegia and hyperreflexia in all 4 extremities. Initial presumptive diagnosis made was transverse myelitis with high cervical cord involvement, and the patient was started on steroid



Figure 1 Magnetic resonance imaging of the cervical spine showing increased T2/STIR signal intensity in the pons, medulla, and upper cervical spine and multiple small flow voids in the dorsal cervicothoracic spine suggestive of dural arteriovenous malformation.

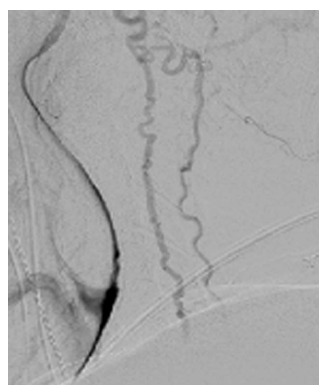


Figure 2 Angiogram showing a Cognard V tentorial dural arteriovenous fistula fed by the left middle meningeal, tentorial branch of the left ICA, and dural branches of the occipital and posterior auricular arteries, with drainage into cervical spinal veins.

therapy. MRI Brain revealed dilated tortuous vessels posterior to the spinal cord between the foramen magnum and the upper thoracic spine. It also showed asymmetric expansion and T2/FLAIR signal abnormality of the left side of the brainstem at the cervicomedullary junction, as well as diffuse expansion and mild diffuse T2 signal abnormality within the cervical and upper thoracic spinal cord (Figure 4).

Cerebral angiogram showed a left transverse sigmoid junction dAVF fed by the left occipital artery (Figure 5). This fistula drained into the superior petrosal sinus, going to the tributaries of the petrosal vein and to the anterior medullary vein. From the anterior medullary vein it then drained down to the anterior spinal and cervicomedullary veins. This venous drainage was thought to be responsible for venous hypertension and the subsequent quadriplegia that the patient was experiencing. This DAVF was successfully embolized with Onyx (Covidien Inc., Mansfield, MA). Three month follow-up angiogram revealed no opacification of the fistula with left occipital artery injection (Figure 6). Clinically the patient had regained complete strength in all 4 extremities and was back at work performing manual labor. The Aminoff-logue scale changed from 7 preintervention, to 0 after embolization

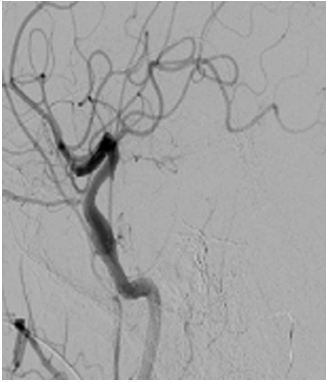


Figure 3 Arteriovenous fistula shown in Figure 2 now successfully embolized with Onyx without any residual filling seen following embolization.

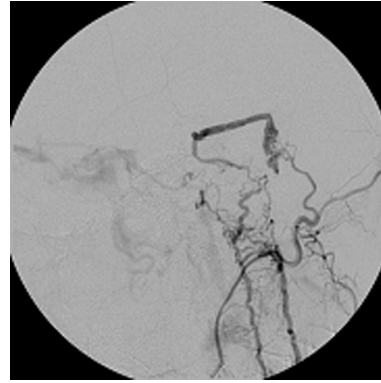


Figure 5 Cerebral angiogram showed a left transverse sigmoid junction dural arteriovenous fistula fed by the left occipital artery.



Figure 4 Magnetic resonance imaging brain and cervical spine showing asymmetric expansion and T2/FLAIR signal abnormality of the left side of the brainstem at the cervicomedullary junction, as well as diffuse expansion and mild diffuse T2 signal abnormality within the cervical and upper thoracic spinal cord.

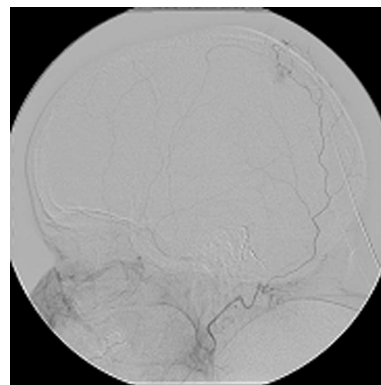


Figure 6 Post onyx embolization angiogram shows no persistent opacification of the fistula with left occipital artery injection.

of dural AV fistula.

DISCUSSION

dAVFs are rare vascular lesions that can come to clinical attention as either intracranial hemorrhage (intraparenchymal, subarachnoid, subdural) or as a consequence of venous hypertension. In the latter situation, presenting symptoms are varied and can include pulsatile tinnitus, dementia, seizures, encephalopathy, parkinsonism, intracranial hypertension, and myelopathy^[4]. Here we have presented two cases of rapidly progressive myelopathy related to dAVFs. All fistulas have one or more feeding arteries, derived from the dural arteries or meningeal branches of cerebral arteries, with venous drainage into a venous sinus, leptomeningeal, or spinal veins.

The classification of intracranial DAVFs has evolved over time, based on venous drainage, natural history, and arterial feeders. Cognard classification divides DAVFs in 5 types (I-V). This classification is based on the direction of dural sinus drainage (antegrade or retrograde), the presence or absence of cortical venous drainage, and venous outflow architecture (nonectatic cortical vein, ectatic cortical vein, or spinal perimedullary vein)^[6,7].

The higher the type, the more likely the DAVF is to be symptomatic as a result of increased venous congestion. Both our cases were classified as Cognard Type V lesions. Type V DAVF usually have aggressive symptoms with progressive myelopathy due to spinal cord venous hypertension. The perimedullary venous drainage can extend down to the thoracic or lumbar levels.

The etiology of DAVFs is not entirely clear. It is generally accepted that DAVFs in adults are acquired. Trauma, prior surgery, sinus thrombosis, and stenosis have been proposed as possible etiologies^[3,4,8,9]. An atypical presentation can lead to delays in accurate diagnosis, as clinicians may first think of other causes of rapidly progressive weakness, such as Guillain Barre syndrome and transverse myelitis. Both the cases we have presented were suspected to be suffering from similar neurological ailments. Only after failure to improve with standard therapy were further investigations conducted and the dAVF discovered on MRI imaging. This goes to underscore the importance of an accurate and timely diagnosis and the value of MRI in the acute setting since delayed diagnosis leads to grave neurologic and functional prognosis^[10].

MRI findings can be subtle and difficult to interpret. These include prominent perimedullary flow voids as well as T2 signal intensity in the brainstem and spinal cord

indicating the presence of venous congestion and edema. Diffuse signal change in the brainstem and spinal cord, with dilated perimedullary veins combined with a presentation of progressive myelopathy should always raise the suspicion of a DAVF^[8]. Cerebral angiography is the gold standard for diagnosis. Arterial feeders to DAVFs can originate from branches of the external carotid artery such as the occipital artery, posterior meningeal artery, middle meningeal artery, as well as meningeal branches of pial arteries^[9,11,12]. When the arterial phase of spinal angiography is negative, an extended observation of the venous phase should be performed looking for venous stagnation which is a sign of venous hypertension.

The optimal management strategy for DAVF is still controversial. It is recommended that DAVF be managed at a center with multidisciplinary experience in endovascular therapy, microsurgery and radiosurgery. Although both of our patients were successfully treated endovascularly, there is still a major role of conventional microsurgery in selected patients. Surgical therapy involves ligation and disruption of the arterio-venous fistulous connection, with success rates of 87.5% to 100% reported in literature^[1-3,12]. Embolization with Onyx can be used as a first line therapy in many patients, with reportedly high rates of durable cure and low rates of complications^[13,14]. There is some evidence to suggest that surgical ligation may offer permanent cure without any recurrence compared to endovascular therapy where recurrence may occur^[15].

Endovascular and surgical therapies are associated with significantly improved symptoms once the definitive diagnosis of SDAVF is made, with studies showing significant improvement in patient outcomes measured on the Aminoff-Logue scale. The Aminoff-Logue scale is a disability scale comprising of three subcategories which score the patient on their gait, micturition and bowel control. Literature supports post-treatment improvement particularly in the subcategories of micturition and gait^[16].

In conclusion, DAVFs are rare but aggressive and potentially fatal vascular malformations. Atypical presentation can mimic other more common neurologic disorders delaying diagnosis. Early diagnosis is important in these cases as a prompt intervention can result in great functional outcomes as evidenced by our cases.

COMMENTS

Case characteristics

The authors present two cases of dural arteriovenous fistula presenting as rapidly progressive myelopathy.

Clinical diagnosis

In one instance the patient demonstrated bilateral lower extremity weakness, whereas the second patient had quadraparesis with significant weakness of all 4 extremities.

Differential diagnosis

Differential diagnosis includes Guillain Barre syndrome, transverse myelitis and various other demyelinating illnesses.

Imaging diagnosis

MRI showed T2 signal change in the spinal cord as well as prominent dorsal flow voids. Cerebral angiogram showed the presence of a dural arteriovenous fistula in both cases.

Treatment

Both patients were treated with Onyx embolization that resulted in complete resolution of the dural AV fistula.

Related reports

This is an unusual presentation of tentorial dural AV fistulas which normally present as hemorrhage and not many cases have been reported in the literature.

Term explanation

Dural arteriovenous fistulas are abnormal connection between arteries within the dura mater and veins that normally drain brain tissue.

Experience and lessons

Dural AV fistulas are rare vascular lesions and can be classified using the Cognard classification. This case report highlights two cases of tentorial dural AV fistulas presenting as progressive myelopathy and discusses treatment options available.

Peer review

Very interesting case reports. It is worth to publish.

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Relapsing polychondritis with p-ANCA associated vasculitis: Which triggers the other?

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which raises the possibility of associated hemophagocytic syndrome. In the setting of RP or AAV physicians should always be aware of the possibility of sudden or insidious appearance of the other disease.

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Key words: Relapsing polychondritis; Anti-neutrophil cytoplasmic antibody; Anti-neutrophil cytoplasmic antibody-associated vasculitis; Rapidly progressive glomerulonephritis; Immunosuppressive treatment

Core tip: Relapsing polychondritis (RP) is a rare disease usually diagnosed late when serious symptoms occur. Appearance of renal symptoms significantly increases the possibility of associated associated vasculitis (AAV). We present three cases of RP in whom AAV occurred at different times during the illness. AAV caused rapidly progressive glomerulonephritis (RPGN) in the second and third patient. Aggressive immunosuppression resulted in remission of both RP and AAV. In the RPGN cases dialysis could be discontinued.

Abstract

Relapsing polychondritis (RP) is a rare autoimmune disease with chronic inflammatory/destructive lesions of the cartilaginous tissues. In one third of the cases it is associated with other autoimmune disorders, mostly with anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV). We report three cases of RP with p-ANCA positive AAV. In the first patient RP developed 1.5 years after the onset of AAV. In the others the signs of RP were present before the onset of severe crescent glomerulonephritis. Patients responded well on steroid and cyclophosphamide. In dialysis dependent cases plasmapheresis was also used successfully. During the 2 and 1.5 years of follow up, they were symptom-free, and had stable glomerular filtration rate. The first patient died after four years of follow-up due to the complications of sudden onset pancytopenia,

File I, Trinn C, Mátyus Z, Ujhelyi L, Balla J, Mátyus J. Relapsing polychondritis with p-ANCA associated vasculitis: Which triggers the other? *World J Clin Cases* 2014; 2(12): 912-917 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i12/912.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i12.912>

INTRODUCTION

Relapsing polychondritis (RP) is a rare disease characterized by recurrent inflammatory flares of cartilaginous structures of ear, nose, joints, larynx and tracheobronchial tree^[1-4]. The aetiology of RP is not clearly defined, but the pathogenesis should involve an autoimmune response to cartilage^[5]. About one third of RP cases can be associated with other multi-system diseases, of which



Figure 1 Red and swollen ear of patient 1. The inflammation spares the lobule.

primary systemic vasculitides are the most common. anti-neutrophil cytoplasmic antibody (ANCA) may be present in up to 25% of patients with RP^[6]. Some of these patients show a classical clinical picture of one of the ANCA associated vasculitides (AAV) and polychondritis is usually thought to be a secondary phenomenon^[7-9]. However many RP patients with ANCA positivity do not have any, or only limited, vasculitic symptoms^[6] and the occurrence of RP may precede AAV^[10-12]. It is possible that the development of ANCA could be provoked by RP, as it was seen by us in rheumatoid arthritis patients^[13].

Whatever is the sequence of disease manifestations, the occurrence of renal symptoms significantly raise the possibility of (underlying or secondary) AAV, the need for more aggressive treatment, and indicates worse prognosis^[14-17]. We present three cases of RP in whom AAV occurred at different times during the illness. Two patients developed rapidly progressive glomerulonephritis (RPGN). The aggressive treatment resulted in dialysis independence in both cases.

CASE REPORT

Case report 1

In March 1998 microscopic polyangiitis was diagnosed in a 58 years old male, based on four weeks' history of fever, anaemia, purpura, arthralgia, episcleritis, axonal neuropathia, and p-ANCA positivity of 38 U/mL (normal < 3 U/mL). Glomerular haematuria, granular casts and mild proteinuria were also present. The serum creatinine was normal, therefore a kidney biopsy was not performed. Renal angiography did not find any aneurysms. Skin biopsy verified small vessel vasculitis. Per os treatment with 1 mg/kg steroid and 2 mg/kg cyclophosphamide resulted in quick resolution of symptoms and p-ANCA negativity, but 2 mo later severe leucopenia and herpes in-

fection of the skin developed, therefore cyclophosphamide was withdrawn. The patient was well on a low dose steroid, but after tapering the dose to 4 mg/d in November 1999 episcleritis reoccurred. Painful swelling and redness of both auricles with sparing of the ear lobe had also developed (Figure 1). Auricular polychondritis spontaneously diminished, but in the next months it relapsed twice. Less severe inflammation of the nose bridge was also present. Based on these clinical symptoms the diagnosis of relapsing polychondritis was established. ANCA remained negative and no other signs of systemic vasculitis reoccurred. Increased steroid dose and azathioprine resulted in remission of polychondritis, therefore six month later azathioprine was withdrawn and only 4-8 mg of methylprednisolone was applied. In June of 2002 fever, weakness and purpura reoccurred. Severe thrombocytopenia (24 G/L), leucopenia (1,2 G/L) and anaemia (Hb 78 g/L) were also present. Bone marrow biopsy showed hyperregenerative cell lines but also a delay in cell maturation thus leading to pancytopenia. Occasionally macrophages containing red blood cell fragments within their cytoplasm were also present. No primary haematological disease was seen and ANCA was negative. Pulse steroid treatment was given resulting in quick improvement of pancytopenia. In August 2002 pancytopenia suddenly reoccurred and the patient died within 24 h after admission into another institution. No autopsy was performed.

Case report 2

A 63-year old woman was admitted to our Department in July 2012 with two months' history of 6 kg weight loss, fatigue, subfebrility, elevated C-reactive protein and normocytic anaemia. She had renal failure as well and needed urgent haemodialysis (serum creatinine 1040 μ mol/L). Urinalysis disclosed proteinuria and glomerular haematuria, ultrasound showed normal size kidneys. Rapidly progressive glomerulonephritis was suspected. The renal biopsy demonstrated pauci-immune necrotizing glomerulonephritis with fibrocellular crescents being present in 70% of glomeruli (Figure 2). She had elevated anti-MPO titer: 21 U/mL (normal < 5 U/mL). The diagnosis of ANCA associated systemic vasculitis was established. Typical signs of auricular chondritis were also present, her ears were tender and had cauliflower appearance. She complained of dizziness, hearing loss, and compromised smell. Her bilateral mixed hearing loss was diagnosed 8 years earlier. In the recent years she had migrating transient polyarthralgia, recurrent nasal obstruction and red eyes, but medical consultation was not sought except due to hypertension in 2010. These signs and symptoms led to the diagnosis of relapsing polychondritis. Pulse steroid of 3 \times 1 g was given and five sessions of plasmapheresis were performed. Treatment resulted in immediate resolution of the inflammatory symptoms. Maintenance immunosuppression was continued in a dose of 0.5 mg/kg per day prednisolone and 1.5 mg/kg per day cyclophosphamide per os. Renal function improved, in February 2013 dialysis could be discontinued, cyclophosphamide was

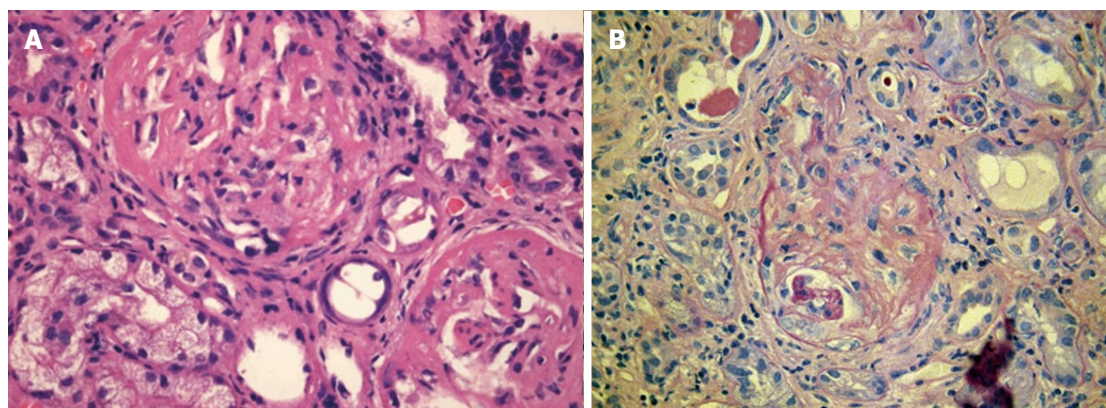


Figure 2 Renal biopsy of the second patient: extensive fibrocellular crescents in the glomeruli. A: Haematoxylin-eosin $\times 40$; B: Periodic acid-Schiff $\times 40$.

withdrawn. Prednisolone was stopped in June 2014 (estimate GFR 20 mL/min per 1.73 m², proteinuria 0.5 g/L). During the 2 years of follow-up, no relapse of vasculitis or polychondritis was observed. Anti-MPO level remained negative, her hearing and the shape of her ears returned to normal.

Case report 3

A 56-year-old woman - with a ten year history of hypertension - was referred to our Department in October 2012 due to RPGN requiring dialysis. ANCA associated glomerulonephritis was established based on > 100 U/mL anti-MPO (normal < 5 U/mL) and pauci-immune glomerulonephritis (fibrocellular crescents in 10 of 32 of glomeruli) seen in the kidney biopsy. In recent years she repeatedly experienced hoarseness, sore throat, laryngotracheal pain, swollen, tender, painful ears and low grade fever. Symptoms sometimes disappeared spontaneously, sometimes she was treated with antibiotics and analgesics. She also had migrating arthralgia. Based on these signs preceding polychondritis was also diagnosed. Before the start of her complaints she punctured her finger while vaccinating rabbits against myxomatosis. At that time (September 2011) laboratory tests revealed normal renal function without proteinuria and haematuria. In September 2012 her throat and ear complaints reoccurred accompanied by fever, fatigue, weight loss, macroscopic haematuria and oliguria. Considering this acute episode she was started on the following therapy: pulse prednisolone (4×0.5 g), plasmapheresis (5 session), and 1.5 mg/kg oral cyclophosphamide. The treatment resulted in resolution of the inflammatory symptoms, renal function improved, and dialysis could be discontinued. Anti-MPO level decreased to 16 U/mL. The patient was discharged in good condition with 0.8 mg/kg per day methylprednisolone and 1.5 mg/kg per day cyclophosphamide. After three weeks she needed admission to our intensive care unit due to high fever, repeated convulsions, agitation and unconsciousness. ANCA titer was normal, renal function has not deteriorated, and her ears and throat did not show signs of inflammation. Therefore cerebral symptoms were suspected not caused by a vasculitic

episode but rather by an immunosuppression-related cerebral infection. Herpes encephalitis was diagnosed by liquor herpes simplex virus polymerase chain reaction positivity. Intravenous acyclovir and immunoglobulin was administered, the steroid dose increased and cyclophosphamide discontinued. This therapy resulted in a slow but full recovery regarding cerebral symptoms. The steroid was gradually tapered and stopped after 1 year. There was no relapse of polychondritis or vasculitis during the 18 mo follow-up, eGFR stabilized about 20 mL/min per 1.73 m², there was no proteinuria, and ANCA tests were negative.

The most important laboratory findings are presented in the Table 1.

DISCUSSION

We present three cases of ANCA associated vasculitis, who also merit the diagnostic criteria for RP. They are not exceptional cases, because vasculitis can be seen in 14% of RP patients^[1], ANCA positivity in up to 25% of RP patients^[6]. The annual incidence of AAV and RP (which is about 10-30/million and 3.5/million population respectively) makes it unlikely that these cases represent simple coincidence.

About one third of RP cases can be associated with other autoimmune diseases, of which vasculitis is the most common^[1-4]. All types of vasculitis were already reported with RP, including microscopic polyangiitis^[10,14], polyangiitis with granulomatosis^[18], eosinophil granulomatosis with polyangiitis^[19], among them most frequently AAV. In our three cases microscopic polyangiitis was diagnosed based on the general and renal signs of systemic vasculitis, the absence of eosinophilia, allergic rhinitis/asthma or sinusitis/otitis. The p-ANCA, anti-MPO positivity is also characteristic for MPA.

There are no specific clinically applicable tests to confirm the diagnosis of RP. Antibodies against type II collagen and matrilin-1 (cartilage matrix protein prominent in tracheal, auricular, and nasal cartilages) can be detected in sera of patients with RP, however their sensitivity and specificity is very low^[5]. Therefore the diagnosis of RP is based on clinical signs^[20]. Currently the diagnosis of

Table 1 Laboratory findings in relapsing polychondritis patients with systemic vasculitis

	Case 1, male 58 yr 03, 1998	Case 2 female 56 yr 7, 2012	Case 3, female 63 yr 10, 2012
Proteinuria (g/d)	0.2	1.03	4.7
Haematuria (vvt/hpf)	15	516	107
Hb (g/L)	95	63	69
CRP (mg/L)	209	101	103
UN (mmol/L)	6.3	41	28
Creatinine (μmol/L)	105	1040	534
GFR (mL/min per 1.73 m ²)	> 60	3	7
Anti-MPO ab (U/mL)	38 (normal < 3)	21.8 (normal < 5)	> 100 (normal < 5)

CRP: C-reactive protein; GFR: Glomerular filtration rate.

RP requires the presence of a proven inflammation in at least 2 of 3 of the auricular, nasal, or laryngotracheal cartilages, alternatively, a proven inflammation in one of the above cartilages and two other signs including ocular inflammation, hearing loss, vestibular dysfunction, or seronegative arthritis^[21].

In our cases the auricular chondritis was the diagnosis-raising sign, but there were other signs in every case to meet the diagnostic criteria of RP. In the first patient recurrent polychondritis developed 1.5 years after the onset of typical vasculitis (neuropathy, purpura, haematuria) when the steroid dose was tapered off. This supports the concept, that RP is a secondary phenomenon of underlying AAV. However, at that time vasculitis was not active, furthermore ANCA was negative. This observation is counter to the findings outlined in a recent case report^[9].

In Case 3 the ear and throat symptoms preceded the vasculitis by one year. Her symptoms started after an accidental needle-puncture while vaccinating rabbits against myxomatosis. It is possible that the attenuated *Myxoma virus* was the trigger activating the immune system by molecular mimicry. In Case 2 AAV and auricular chondritis occurred at the same time, but her hearing loss preceded them by eight years. From that time she had recurrent auricular, nasal and ophthalmological symptoms, which raises the possibility of RP. The diagnosis of RP is difficult in the early stage because the incidence of each symptom is less than 50% at the onset^[1]. The diagnosis is usually delayed by 3 years^[22] but the delay can be as long as 10 years^[23]. Extremely precise case history and clinical evaluation is needed. In this case the biopsy of an involved cartilage could help. Biopsies, however, often show only nonspecific granulation tissue, so the pathognomonic findings for RP may be not be easy to obtain^[24].

In spite of the fact that glomeruli do not contain type II collagen, renal involvement was reported in 29/129 cases in the Mayo Clinic study^[17]. Haematuria was the most frequent abnormality occurring in 26% of 337 patients^[1]. It was observed in all our cases, indicating a proliferative glomerulonephritis. Rapid decline in glomerular filtration rate (GFR) was seen in two cases raising the sus-

picion of pauci-immune crescentic glomerulonephritis, which was verified by a kidney biopsy. This type of glomerular lesion is diagnostic for AAV-s, even if ANCA is not present. In the early phase of kidney damage only focal segmental glomerular necrosis can be present. These lesions were the most frequently observed pathological finding in RP and rose the suspicion of vasculitis even decades earlier when ANCA was not yet available^[14-17]. Less frequently other types of glomerulonephritides, such as IgA nephropathy^[25], membranous nephropathy^[26] had also been reported. We think that these lesions could not to be linked to RP. When renal signs appear it is very important to differentiate renal vasculitis from other causes: e.g., membranous nephropathy could be caused by non-steroid anti-inflammatory drugs, used for the treatment of arthralgia in RP.

Renal vasculitis indicates a worse prognosis and the need for more aggressive immunosuppressive treatment. Due to the poor response of AAV to steroids alone, first-line regimes used in patients with RP/AAV overlap should include additional cyclophosphamide or other immunosuppression. Our patients responded well on steroid and cyclophosphamide treatment. In dialysis-dependent cases we combined it with plasmapheresis. This regime resulted in dialysis independence in spite of the advanced histological picture. The patients became symptom-free both regarding RP and AAV. They have severely decreased GFR, which could have been prevented had they been referred to us earlier. The first patient died after four years of follow-up due to the complications of sudden onset pancytopenia. No primary haematological disease was seen on bone marrow biopsy. Therefore it was thought to be a result of a flare of the underlying autoimmune disease, which was supported by the fact that pulse steroid treatment had been effective. The presence of hemophagocytosis and the recurrence of pancytopenia with sudden respiratory failure raise the possibility of hemophagocytic syndrome. Its association with adult onset autoimmune disease has recently gained attention^[27,28]. The association of RP and AAV can lead to critical conditions and treatment needs to be initiated promptly and undertaken by an experienced team. RP patients need a regular and prolonged follow up for renal symptoms and ANCA-s as well.

Recommendation

In any case of RP or AAV physicians should be aware of sudden or insidious appearance of the other disease.

COMMENTS

Cases characteristics

A 58-year-old male diagnosed with microscopic polyangiitis experienced painful swelling and redness of both auricles, a 63-year-old woman had renal failure, tender and cauliflower-like ears, a 56-year-old woman -with a history of relapsing polychondritis-presented with rapidly progressive glomerulonephritis.

Clinical diagnosis

Swelling and redness of both ears, arthralgia, red eyes, hearing loss, tracheo-bronchial pain pointed to relapsing polychondritis, while purpura, general (fever,

fatigue, weight loss), and renal (haematuria, oliguria) symptoms to vasculitis.

Differential diagnosis

Microscopic polyangiitis, polyangiitis with granulomatosis, eosinophil granulomatosis with polyangiitis, other vasculitides, systemic lupus erythematoses, other causes of rapidly progressive glomerulonephritis (RPGN) can be considered.

Laboratory diagnosis

High C-reactive protein, anaemia, p-anti-neutrophil cytoplasmic antibody/anti-MPO positivity, haematuria, proteinuria, elevated serum creatinine, decreased glomerular filtration rate (Table).

Imaging diagnosis

Chest X-ray was unremarkable, abdominal ultrasound showed normal size kidneys.

Pathological diagnosis

Skin biopsy of the first patient showed small vessel vasculitis, renal biopsy of the other two patients was consistent with pauci-immune crescentic glomerulonephritis.

Treatment

Immunosuppressive treatment with steroid, cyclophosphamide, azathioprine; in the RPGN cases plasmapheresis was the specific medication.

Related reports

There are only scattered case reports about the association of relapsing polychondritis (RP) and associated vasculitides (AAV).

Term explanation

Pauci-immune glomerulonephritis is diagnosed based on extensive extracapillary proliferation (leading to crescent formation) and necrotic lesions in the capillary tuft with negative immunofluorescent and electron microscopic finding.

Experiences and lessons

In any case of RP or AAV physicians should be aware of sudden or insidious appearance of the other disease.

Peer review

It is properly written article on case series of relapsing polychondritis and vasculitis.

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Gastric trichobezoar associated with perforated peptic ulcer and *Candida glabrata* infection

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picture, at no point was the presence of a giant bezoar at gastric level suspected, specifically a trichobezoar. The emergency abdominal and pelvic ultrasound revealed only unspecific signs of perforated hollow viscus. Diagnosis was therefore made intraoperatively. A complete gastric trichobezoar was found with gastric perforation and secondary peritonitis. The peritoneal fluid culture revealed *Candida glabrata*.

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Key words: Bezoar; Postpartum period; Acute abdomen

Core tip: This case report describes the presentation of a gastric trichobezoar as an acute abdomen in puerperal woman with a detailed clinical description and images. The discussion provides an extensive analysis on the various types of treatments that exist today and their results, as well as evaluating its association with specific psychiatric diseases, and the finding of *Candida* infection in the patient evolution.

Losada Morales H, Huenchullán Catalán C, Arriagada Demetrio R, Espinoza Rivas M, Castagnoli Parraguez N, Alanis Alvarez M. Gastric trichobezoar associated with perforated peptic ulcer and *Candida glabrata* infection. *World J Clin Cases* 2014; 2(12): 918-923 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i12/918.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i12.918>

Abstract

Bezoars are accumulations of human or plant fiber located in the gastrointestinal tract of both humans and animals. Patients remain asymptomatic for several years, and the symptoms develop as these accumulations increase in size to the point of obstruction or perforation. We report the case of a 21-year-old patient at 10 d postpartum, who presented with acute abdomen associated with sepsis. Given the urgency of the clinical

INTRODUCTION

The trichobezoar is a rare medical condition composed of a mass of hair in the proximal gastrointestinal tract, which can cause obstruction, and almost exclusively affects young women^[1,2]. Its prevalence ranges from 0.06% to 4% in the general population^[3]. It is considered a re-

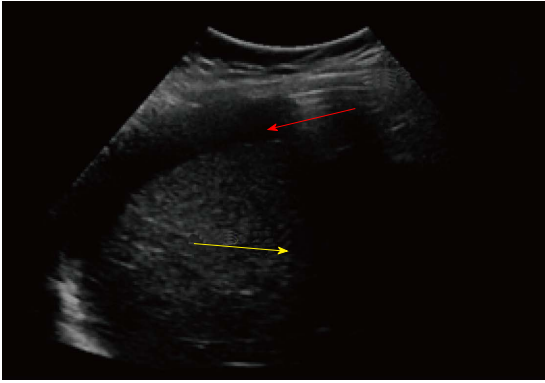


Figure 1 Abdominal ultrasound at epigastric level shows moderate amount of free fluid (yellow arrow) and reverberation artifact immediately under the abdominal wall compatible with intraperitoneal free air (red arrow).

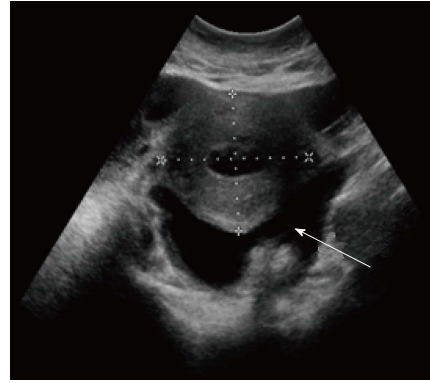


Figure 2 At pelvic level, enlarged uterus is observed associated with free fluid, which presents echogenic material within suggestive of pus (white arrow).

sult of trichotillomania, a psychiatric disorder linked to compulsively removing hair from the head and body in general^[4]. When hair is ingested, it resists digestion and peristalsis, which is why it accumulates in the folds of the gastric mucosa. Mostly it remains confined to this level; but on some occasions it passes the pylorus, reaching the jejunum, ileum and even the colon. This condition was described for the first time by Vaughan *et al*^[5] in 1968, and was called Rapunzel syndrome. The aim of this work is to report the clinical case of a patient with acute abdomen involving perforated hollow viscus associated with sepsis during the post-Cesarean period, who presented with a giant trichobezoar intraoperatively, and to review the clinical presentation, study through imaging, risk factors, complications and treatment.

CASE REPORT

Patient, 21 years of age, female, with a history of insulin resistance with no current pharmacological treatment. She came to the emergency room of the Clínica Alemana Temuco, Chile ten days after an uneventful Cesarean section, referred from the hospital in Angol due to acute pain in her left side with 24 h of evolution, colicky in nature and with an intensity of 10/10 on the pain scale, without radiation, associated with sweating and involvement of her general state. In the initial evaluation, she was described as endomorph (BMI: 26), with altered vital signs in the range of systemic inflammatory response syndrome: blood pressure 124/80 mmHg; mean blood pressure 87 mmHg; heart rate 150 beats per minute in sinus rhythm; temperature 36 °C; respiratory rate 21 breaths per minute; oxygen saturation 98% with 3 L/min of oxygen *via* nasal prongs. On physical examination she was alert, focused and responsive (Glasgow Scale: 15 points), pale in skin and mucosa, vesicular murmur reduced in both lung fields, and abdomen distended with no sounds, yielding to the touch with diffuse tenderness with signs of peritoneal irritation. The laboratory examinations on admittance were as follows: hematocrit 34.7%; leukocyte count: 16100 K/uL; platelets: 984000

K/uL; erythro sedimentation rate: 41 mm/h; C-reactive protein: 502 mg/L; creatinine: 1.52 mg/dL; uremia: 64 mg/dL; prothrombin percentage/thromboplastin time: 55.7%/42.1 s; INR: 1.3; sodium: 134 mmol/L; potassium: 5.5 mmol/L; chlorine: 105 mmol/L. The abdominal-pelvic ultrasound taken in the emergency room revealed a moderate amount of free fluid and pneumoperitoneum; the uterus was enlarged due to purulent fluid at the bottom of the Pouch of Douglas (Figures 1 and 2). The patient was admitted to the ICU with diagnoses of: (1) Acute abdomen; (2) Systemic inflammatory response syndrome (SIRS); (3) Early postpartum 10th day Cesarean section; and (4) Acute renal dysfunction.

Support began with conservative therapy: zero regimen, oxygen therapy and resuscitation with fluids. Follow-up tests 3 h later: hematocrit: 23.5; leukocytes: 10500; platelets: 735000. The patient received antibiotic prophylaxis (2 g ceftriaxone and 500 mg metronidazole) and a transfusion of 2 UI of deep frozen fresh plasma. Through imaging, the patient was considered to be in post-Cesarean period with acute abdomen, abdominal sepsis and possible perforated hollow viscus. An exploratory laparotomy was performed, which found purulent fluid in 4 quadrants, a left subphrenic abscess, cecal appendix with the end phlegmonous and ulcerated, two perforated gastric ulcers, one in the anterior wall and another in the posterior wall, with a collection of gastric fluid in the omental bursa, and complete gastric trichobezoar involving the stomach and duodenum (weight: 1090 g) (Figure 3). The technique used was an anterior gastrotomy and bezoar extraction (Figures 4-6), closing the wound, including the ulcer, with a running stitch of 3-0 polydioxanone, single-layer suture and running stitch with 2-0 silk. Closing the ulcer in the posterior wall was done with 3-0 polydioxanone and an omental patch, fixing it with 3-0 silk.

The patient evolved favorably in the early postoperative phase, but at 3 d she began with a fever up to 38.9 °C, for which empirical antibiotic treatment was introduced. She remained hospitalized 25 d and in total and four days in intensive care unit. Peritoneal fluid culture



Figure 3 Trichobezoar extracted weighing 1.09 kg.

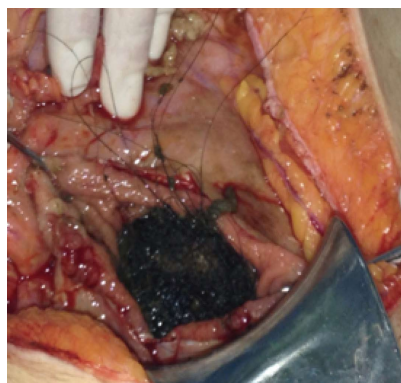


Figure 5 Gastric opening for extraction of trichobezoar.

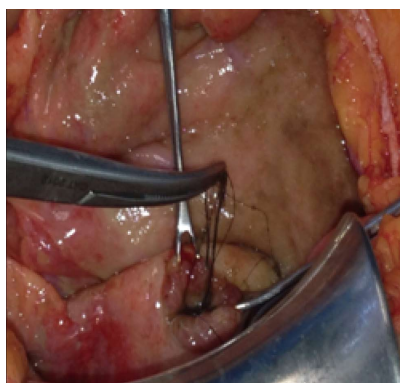


Figure 4 Finding of hair through perforated ulcer.

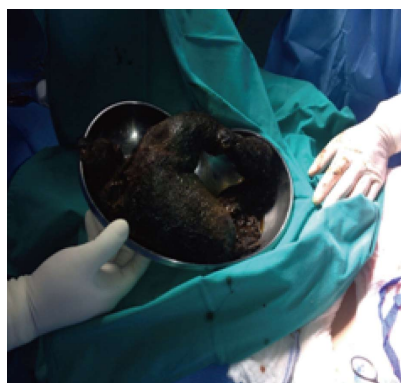


Figure 6 Trichobezoar mass taking the shape of the stomach and part of the duodenum.

was positive for *Candida Glabrata*; therefore, infectology assessed the case and decided to initiate anti-fungal drugs due to the presentation in the case of a hollow viscus rupture in conjunction with a bezoar. During hospitalization a psychiatric evaluation, during an interview of the patient and her mother, revealed that she had exhibited trichotillomania and trichophagia daily since the age of 12. The behavior decreased during pregnancy, and she was therefore diagnosed with moderate anxiety disorder with trichotillomania and inactive trichophagia. The methylene blue test was negative on the seventh postoperative day. Antibiotic therapy with ertapenem in addition to caspofungin was completed after two weeks. Evolution was good, with a good response, so discharge was given, completing oral anti-fungal treatment with voriconazole for three weeks.

DISCUSSION

A bezoar is defined as a mass of ingested foreign material accumulated in the digestive tract. It means “protection against poison” or antidote, given its use for curative purposes and even superstitions associated with good luck^[6,7]. The first recorded case was in 1779, when Baudamant described the presentation in a woman^[8]. Bezoars are classified into 5 groups according to the substance that comprises it: phytobezoar, pharmaco-bezoar, trichobezoar, lactobezoar and foreign body bezoar^[9].

Bezoars are described as occurring in 1% of the population, associated mainly with gastric disorders such as hypomotility, hyposecretion with hypochlorhydria, and a history of resection^[10]. In one retrospective study, 87 cases of intestinal bezoars were presented, in which diagnosis was made using the clinical history plus an endoscopy. The results included the presence of a prior surgery in 76 of the cases. In 3% of the cases, the most widely used technique was the bilateral truncal vagotomy with a pyloroplasty (75.8%). Other factors to consider would be an excess of plant fiber ingestion in 39.5%, and alterations in teething and chewing in 24%^[11].

In our case it was more important specifically to investigate the presence of trichotillomania, a disorder listed in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders, in the section on Obsessive-Compulsive and Related Disorders^[12]. Although its prevalence and comorbidity have not been clearly determined, different series of cases describe from 5% to 30% of patients with trichotillomania as having associated trichophagia^[13-15], while 1% to 37.5% of these will develop a trichobezoar^[13,15-17].

The clinical presentation generally occurs with symptoms and signs of acute abdomen and intestinal obstruction. This includes abdominal pain, nausea, bilious vomiting, hematemesis, anorexia, early satiety, weakness, weight loss and an abdominal mass^[18-20]. Associated systemic

complications include anemia, due either to nutritional deficit or gastrointestinal bleeding, and gastric ulcers have been documented in cases of gastric bezoars^[21]. Imaging, such as ultrasound, can be useful in the detection of an abdominal mass, although computed tomography is more precise in assessing the characteristics of a bezoar and will allow the additional identification of bezoars in other places in the gastrointestinal tract. The diagnosis is established by endoscopy^[2,18,22], and sometimes this can be effective as a treatment as we will analyze later.

In terms of surgical approach, this is where most of the debate lies with respect to management. Laparotomy, laparoscopy, endoscopic removal and even chemical dissolution in the case of phytobezoars have all been proposed. The choice is made on the basis of the size and composition of the bezoar^[6,23].

The endoscopic removal of a trichobezoar has not been standardized, and a variety of techniques have been described in the literature, among which the following have been emphasized: fragmentation and removal with forceps, polypectomy snare, hydrolysis, neodymium-doped yttrium aluminum garnet laser, mechanical lithotripsy, electrohydraulic lithotripsy or extracorporeal shock wave lithotripsy^[9,24-27]. The first report of the successful endoscopic removal of a trichobezoar was for one that weighed 55 g^[28]. An analysis of case reports where endoscopy was used as a first approach revealed a success rate of only 5%^[29]. The difficulties of this technique are due to an unsuccessful fragmentation that might be explained by the size trichobezoars can reach, as well as by the density and hardness of the formed mass^[30,31]. The repeated introduction of the endoscopy to achieve removal of all the fragments can cause esophagitis, pressure ulcers and even esophageal perforation^[32]. Even in cases like ours of large trichobezoars, the attempt at fragmentation may allow the bezoars to migrate beyond they pylorus and cause an intestinal obstruction. The search for satellites in the intestine is not optimal with endoscopy, and their removal is impossible. Thus the role of endoscopy has advanced more towards diagnostic management than treatment. It allows us to differentiate, in the context of a gastric mass of an unknown nature, between a trichobezoar and foreign bodies that can be extracted or fragmented *via* endoscopy^[33].

As far as surgical removal is concerned, it is important to differentiate between the classic and the laparoscopic approaches. The first report of a successful result of laparoscopy for a trichobezoar was published in 1998 regarding a 7-year-old girl^[21]. To date the case reports endorsing this technique have been few, mainly in the pediatric population, limited mainly by the size and the presence of satellites in the intestine^[29,30,32,34-37]. The combination of techniques has been used, implementing fragmentation laparoscopically and removal endoscopically^[38]. The advantages of laparoscopy as it relates to a trichobezoar are a lower rate of postoperative complications, a reduced hospital stay, and a better cosmetic result. The disadvantages are a longer operating time, greater

complexity in the review of the intestine in search of satellites, and the risk of contaminating the abdominal cavity with hair fragments^[39].

The laparotomy has been successful in most cases: over 100 cases of successful results with this technique have been described^[40]. Complications with this technique are described in 12% of the cases^[40], and these include intestinal perforation during removal of the trichobezoar^[41,42], infection of the surgical wound^[43], pneumonia, and paralytic ileum^[44].

Another interesting point to analyze in our case is the *Candida Glabrata* infection. One study reported on the cases presented for one year with a diagnosis of peritonitis due to a perforated peptic ulcer. There were 62 cases in all, of which 23 (37.09%) had peritoneal fluid cultures that tested positive for *Candida*, ten (16.12%) for isolated bacteria and the rest were negative. That analysis concluded there were no significant risk factors for developing the different species. In addition, they marked an important prognostic factor with an up to 21.7% likelihood of mortality for the case of *Candida* peritonitis, compared to the results for bacterial peritonitis and negative cultures, which were 0% and 3.4% respectively. The specific case of *Candida Glabrata* peritonitis was second in frequency (13.04%) in this series, surpassed by *Candida Albicans* peritonitis with 78.26%^[45]. Other reports associate the gastric mycotic infection as a factor related to gastric perforation^[46]. This point emphasizes the importance of taking a peritoneal fluid culture in patients with secondary peritonitis.

After the anti-fungal treatment, the patient in our report presented a good clinical evolution and was discharged with no associated morbidity.

COMMENTS

Case characteristics

Abdominal pain in a puerperal woman associated with systemic inflammatory response syndrome.

Clinical diagnosis

Acute abdomen.

Differential diagnosis

The principal differential diagnosis was the complications of previous cesarean section, as an uterine rupture or large bowel perforation.

Laboratory diagnosis

Laboratory signs of systemic response.

Imaging diagnosis

Pneumoperitoneum and free fluid suggestive of pus.

Pathological diagnosis

Trichobezoar.

Treatment

Anterior gastrotomy and bezoar extraction.

Related reports

Psychiatric diseases in relation with trichobezoar.

Term explanation

Association with *Candida Glabrata* infection.

Experiences and lessons

The authors need a high level of diagnostic suspicion, and use the anamnesis to find trichotillomania in antecedents to supply our diagnosis.

Peer review

The case-report is interesting and so is the complication and super infection.

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Rare entity: Ectopic liver tissue in the wall of the gallbladder - A case report

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bladder, gastrohepatic ligament, adrenal glands, and esophagus. It is usually clinically silent and found incidentally. Ectopic hepatic tissue carries an increased risk of malignant degeneration to hepatocellular carcinoma. It should be discovered and removed by the surgeon to prevent a higher risk of complications and malignant transformation.

Arslan Y, Altintoprak F, Serin KR, Kivilcim T, Yalkin O, Ozkan OV, Celebi F. Rare entity: Ectopic liver tissue in the wall of the gallbladder - A case report. *World J Clin Cases* 2014; 2(12): 924-926 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i12/924.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i12.924>

Abstract

Ectopic liver tissue (ELT) is a rare condition, which is usually not diagnosed preoperatively, but coincidentally during abdominal surgery. While the location of ELT can vary, it is usually localized on the gallbladder wall or in close proximity. ELT is associated with various complications, a major complication being extrahepatic hepatocellular carcinoma. A 59-year-old female underwent elective surgery for chronic cholecystitis with stones. During laparoscopic exploration, a 2-cm-diameter ELT was detected in the anterior gallbladder wall and a laparoscopic cholecystectomy was performed. The case is presented due to the rare nature of ELT and as a reminder of ELT-related complications.

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Key words: Ectopic liver tissue; Laparoscopic cholecystectomy; Hepatocellular carcinoma

Core tip: Ectopic hepatic tissue is a rare condition and it has been reported in several sites, such as the gall-

INTRODUCTION

Congenital localization anomalies of the liver are rare, and can be classified into two major subgroups: those that are connected to the main liver tissue mechanically, and those that are not^[1]. Ectopic liver tissue (ELT) is a subtype that is not connected to the main liver tissue, and it can be present in any intra-abdominal or supradiaphragmatic location. The most frequent intra-abdominal location is the gallbladder, and ELT is generally identified by recognizing extra tissue on the gallbladder wall that is of the same color as liver tissue^[2].

First defined in 1922, ELT can vary in size, from microscopic scales to 3 cm in diameter. Despite its small size, since it is liver tissue histopathologically, it is prone to parenchymal diseases of the liver, including carcinoma development. Due to its small size, ELT is generally not noticed during routine radiological examinations; however, when noticed, it might be necessary to differentiate it from various conditions, including gallbladder cancer^[3].

Here, we present a case of ELT on the gallbladder wall, which was detected during surgery, and review the relevant literature.

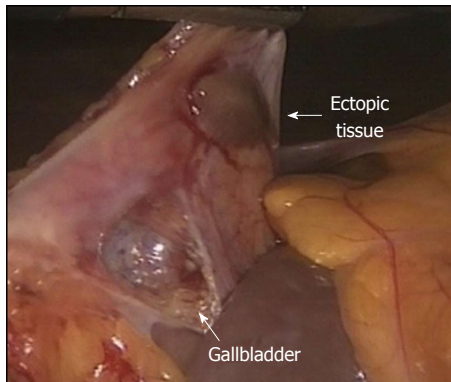


Figure 1 Laparoscopic exploration: A 2 cm × 1 cm tissue mass located at the gallbladder wall, which had the same color as the liver.

CASE REPORT

A 59-year-old female presented to the general surgery clinic with occasional epigastric pain and dyspeptic complaints for 2 mo. She had no history of systemic disease, previous abdominal surgery, or regular medication use, and her physical examination and laboratory tests were normal. Abdominal ultrasonography (USG) revealed a large number of millimeter-size stones in the gallbladder lumen, and she was scheduled to undergo an elective cholecystectomy. Laparoscopic exploration revealed a 2 cm × 1 cm tissue mass located in the fundus of the anterior gallbladder wall, which had the same color as the liver (Figure 1). The patient was diagnosed as having ELT on the gallbladder wall, and underwent a laparoscopic cholecystectomy (Figure 2). She did not have any postoperative problems, and was discharged on the second day. Following histopathological examination, the tissue attached to the gallbladder wall was confirmed to be ELT. The specimen examination showed that it was a truly ectopic liver and was not connected to the mother liver. The patient has been followed without any problems for 5 mo.

DISCUSSION

Ectopic liver tissue is a rare condition that is usually detected coincidentally during a post-mortem examination or abdominal surgery. According to the literature, the incidence of ELT at laparoscopy or laparotomy is 0.24%-0.47%^[4]. In a 5500-case autopsy series, the incidence of ELT was 0.05%^[5], while its incidence was 0.47% in a 1060-case laparoscopic surgery series^[6]. During the past 5 years, 5000 patients underwent abdominal surgery at our clinic for various reasons and ELT was observed in a single patient (0.02%), the case presented here.

The most frequent localization of ELT is the gallbladder, although other sites have been reported, including the adrenal glands, pancreas, spleen, falciform ligament, pylorus, umbilicus, retroperitoneum, thorax (intrapleural/extrapleural), and pericardium^[2]. ELT is generally asymptomatic, but it can present with recurrent abdominal pain due to torsion, hemorrhagic necrosis, or rupture, or with pressure symptoms due to mass formation as a conse-



Figure 2 Laparoscopic cholecystectomy material with ectopic tissue.

quence of malignant degeneration^[7]. We believe that our patient's non-specific complaints were due to cholecystopathy, and not associated with the ELT, because there was not pathological appearance of ELT such as torsion, necrosis.

Various theories have been presented to explain the development of ELT at different sites, and ELT can be seen together with biliary atresia, caudate lobe agenesis, omphalocele, and certain congenital cardiac anomalies^[8]. We did not observe any comorbid anomalies in our case. It is a truly ectopic liver and is not connected to the mother liver. The ectopic liver tissue presents resulting from liver tissue migration to fundus of gallbladder during embryogenesis.

Ectopic liver tissue is not noticed during radiological examinations, as it is generally asymptomatic, rare, small in size, or the examiner is not aware of this entity. The diagnosis of ELT should be considered when a soft-tissue mass is detected on the gallbladder wall using USG or computed tomography. Color Doppler USG and angiography can show the blood vessel feeding the liver. However, the incidence of radiological detection is low, and the number of reported cases for which a preoperative diagnosis has been made is very limited^[2,3]. When noted radiologically, the exact diagnosis is made by showing the presence of hepatic tissue in a percutaneous biopsy; however, this is not a suggested method for diagnosis, due to the bleeding risk and malignant degeneration^[4].

The normal progression of ELT is not known. Since it is liver tissue, it is affected by the same risk factors affecting the liver, and lipid infiltration, cirrhotic changes, chronic active hepatitis, hemosiderosis, metastatic tumors, and hepatocellular carcinoma (HCC) have been reported to develop in ELT^[8]. The development of HCC is the most important condition, and involves a higher risk of neoplastic transformation that is independent of the main liver tissue. The lack of complete functional structure for neoplastic transformation in small ELT, absence of vascular and ductal systems, and possible metabolic insufficiency are believed to contribute to the carcinogenetic process^[4,9].

In conclusion, ELT is a rare condition, and it is difficult to make a radiological diagnosis. When seen during

a surgical intervention, it should be excised because of the possibility of developing a malignancy.

COMMENTS

Case characteristics

Patient presented to the general surgery clinic with occasional epigastric pain and dyspeptic complaints for 2 mo.

Clinical diagnosis

Patient had no history of systemic disease, previous abdominal surgery, or regular medication use, and her physical examination was normal.

Laboratory diagnosis

Laboratory tests were normal.

Imaging diagnosis

Abdominal ultrasonography revealed a large number of millimeter-size stones in the gallbladder lumen.

Pathological diagnosis

Following histopathological examination, the tissue attached to the gallbladder wall was confirmed to be liver tissue.

Treatment

The patient was underwent a laparoscopic cholecystectomy.

Related reports

Laparoscopic exploration revealed a 2 cm × 1 cm tissue mass located in the fundus of the anterior gallbladder wall.

Experiences and lessons

Ectopic liver tissue is a rare condition, and it is difficult to make a radiological diagnosis. When seen during a surgical intervention, it should be excised because of the possibility of developing a malignancy.

Peer review

This paper was concise and well-written.

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Rare multiple fistulas with large saccular aneurysms originating from left anterior descending artery and left main coronary artery

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Abstract

A 49-year-old female patient consulted us for a cardiac evaluation before undergoing colon adenocarcinoma surgery. Three years prior, the patient underwent coronary angiography for dyspnea. The coronary angiography examination revealed a fistula originating from the left anterior descending artery and left main coronary artery, which had soft aneurysmal sacs and most likely drained into the pulmonary artery. Parasternal short axis echocardiography revealed a color flow that could be related to the fistula, but the other echocardiographic findings were normal. The patient did not accept the proposed examination and invasive treatment.

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Key words: Left main coronary artery; Left anterior descending; Fistula; Swinging aneurysmal sacs

Core tip: (1) Acquire technical and surgical skills; (2) Learn the coronary anatomy and its variations; and (3) Learn the methodology of for treating coronary anomalies.

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las with large saccular aneurysms originating from left anterior descending artery and left main coronary artery. *World J Clin Cases* 2014; 2(12): 927-929 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i12/927.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i12.927>

INTRODUCTION

Coronary artery fistulas are rare anomalies that open into the heart chambers, large vessels and other structures by bypassing the myocardial capillary network. Coronary artery fistula is detected in 1.21%-5.60% of all patients undergoing coronary angiography^[1]. Left main coronary artery fistula is extremely rare^[2]. Not all coronary-pulmonary artery fistulas are hemodynamically significant, however some may cause myocardial ischemia, myocardial infarction, congestive heart failure, pulmonary arterial hypertension, aneurysmal fistula rupture and sudden death^[3]. Here we present a case with an angiographically documented coronary artery fistula that originating from the left main coronary artery (LMCA) and dividing into two branches. In addition, another fistula that originated from the left anterior descending artery (LAD) combined with the LMCA fistula and created a new line of fistula, with large saccular coronary sacs, that drained into the pulmonary artery. This type of coronary artery fistula is most rares.

CASE REPORT

A 49-year-old female patient consulted us for a cardiac evaluation before undergoing colon adenocarcinoma surgery. Three years prior, the patient underwent coronary angiography for dyspnea. The coronary angiography examination revealed, a fistula originating from the LMCA which could be interpreted as draining into the pulmonary artery. Upon examining the coronary angiography in detail, in addition to the LMCA fistula, another fistula

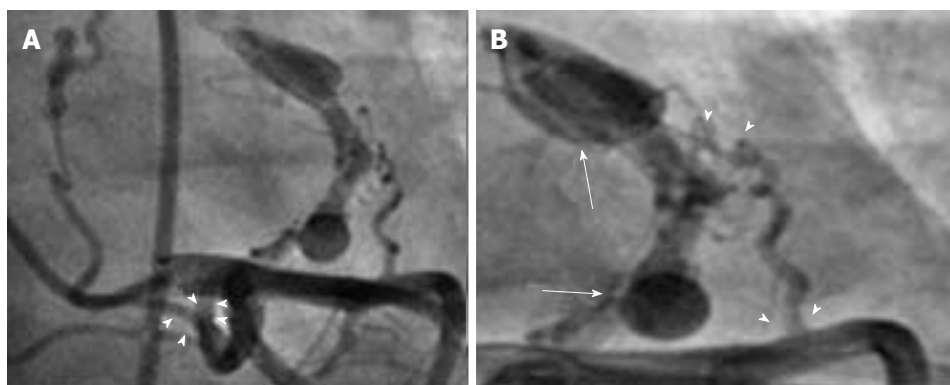


Figure 1 A: The fistula emerging from the left main coronary artery is divided into two branches after the fold (arrowhead); B: An angiographic view showing the combination of a small fistula emerging from left anterior descending artery, the fistula from left main coronary artery (arrowhead) and large sacular sacs on the fistula line (arrow).

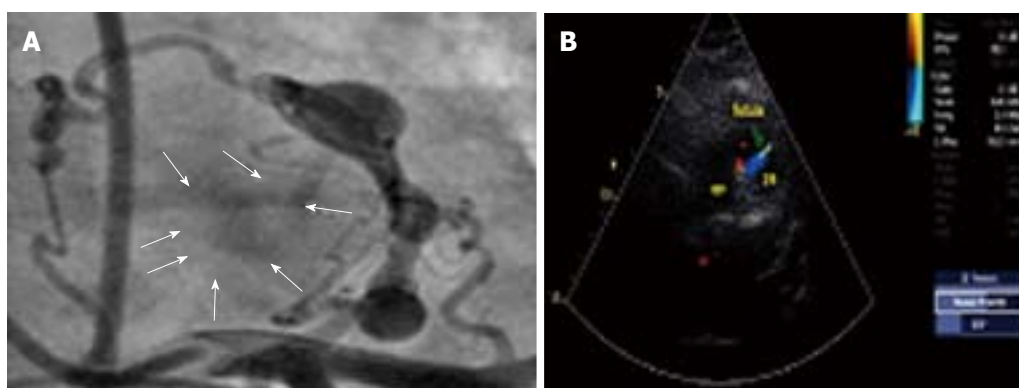


Figure 2 The passage of the contrast agent formed by the fistula (A), parasternal short axis echocardiography revealed the color flow associated with the fistula (B).

emerged from the LAD combined with the LMCA fistula and there was an aneurysmal sac swinging on fistula line (Figure 1). It was thought that the fistula was draining into the pulmonary artery (Figure 2A). Parasternal short axis echocardiography revealed a flow that was most likely caused by the fistula (Figure 2B). There were no regional wall motion abnormalities or systolic dysfunction. Because of excessive fatigue, the stress test was terminated at the end of step 3. The target heart rate and blood pressure were reached and there were no arrhythmia or ST-T wave changes. After further investigations and evaluations were performed, the patient was informed about the possibility of a cardiac operation. The patient did not accept the treatment proposals and further investigation was not planned. The medical risks were explained to the patient and outpatient follow-up was scheduled with full oral medical treatment.

DISCUSSION

Coronary artery fistulas are rare anomalies that open into the heart chambers, large vessels and other structures by bypassing the myocardial capillary network^[1]. The first case of coronary arteriovenous fistula was reported by Krause^[4] in 1865. Coronary artery fistula is detected in 1.21%-5.60% of all patients undergoing coronary an-

giography^[5]. A fistula from the coronary arteries to the pulmonary artery is observed in 0.1%-0.2% of coronary angiographies^[6]. A total of 92% of coronary artery fistulas drain into the right heart chambers and 8% drains into the left heart chambers. The origins of the fistulae are vary, as follows: right coronary artery (50%-60%), left anterior descending artery (25%-42%), both coronary arteries (5%), circumflex artery (18%), diagonal artery (1.9%), marginal arteries (0.7%). Left main coronary artery fistula is extremely rare^[2]. Although coronary artery fistulas are often congenital, they may occur after chest trauma, angiography and bypass surgery^[7].

Coronary artery anomalies often affect hemodynamic parameters. Not all coronary-pulmonary artery fistulas are hemodynamically significant. However some may cause myocardial ischemia, myocardial infarction, congestive heart failure, pulmonary arterial hypertension, aneurysmal fistula rupture and sudden death^[3].

Although two-dimensional echocardiography (transesophageal echocardiography complements two-dimensional echocardiography) is valuable in revealing the fistula, it is operator dependent and because it does not have a good acoustic window, determining the fistula location may be insufficient^[8].

Until recently, using conventional coronary angiography to detect coronary anomalies was the preferred diag-

nostic method. However invasiveness, acquisition plane images, the lack of angiographic projection angle and concerns about the contrast load limit this method^[9]. Multislice computed tomography (MSCT) can better reveal aneurysms, occlusion; as well as the direction of the fistula and its relationship with the cardiovascular structures along the fistula compared with coronary angiography^[8]. MSCT imaging is the recommended technique for the diagnosis and follow up of coronary artery anomalies^[10].

The treatment of asymptomatic patients without significant shunts is still a matter of debate^[11]. The presence of ischemic symptoms or a positive stress test, aneurysmal dilatation with or without mural thrombus and overload of heart chambers due to excessive blood flow are the indications for fistula closure^[12]. In the literature, similar early efficiency, mortality and morbidity rates are observed for both the surgical and transcatheter approaches^[13].

In this case, although the fistula did not affect the hemodynamic parameters and the exercise test for ischemia was negative and because there was a large thrombosed aneurysm sac on the fistula line, transcatheter closure of the fistula was considered. The patient did not accept any attempt at surgical treatment, thus, further examination and treatment could not be performed. The patient was discharged with full oral medical treatment and recommendations. The patient has the risk of sudden death due to aneurysmal sac rupture and thromboembolic event due to aneurysm sac thrombus. Because of progression in the shunt system, a reduction in functional capacity and heart failure may develop. Coronary artery fistula is a possibility because of the degree of shunt or the patient's symptoms; in addition, aneurysmal sacs and thrombus in the fistula line are possible^[5,12].

COMMENTS

Case characteristics

The main symptom of the patient was exertional dyspnea.

Differential diagnosis

The authors took into consideration the diseases, comorbidities and patient's age which causes exertional dyspnea (e.g., coronary artery disease, pulmonary diseases, structural heart diseases, endocrine disorders).

Laboratory diagnosis

The authors made routine laboratory tests including BNP, pro-BNP, serum creatinine, urea, electrolytes, aspartate aminotransferase, alanine aminotransferase, complete blood count.

Imaging diagnosis

Color doppler echocardiography and coronary angiography.

Treatment

Transcatheter and surgical closure; Heart failure treatment; Nitrates, Acetylsalicylic acid, angiotensin converting enzyme inhibitor.

Term explanation

Coronary artery fistula: a sizable communication between a coronary artery

and a chamber of the heart (coronary-cameral fistula) or any segment of the systemic or pulmonary circulation (coronary arteriovenous fistula).

Experiences and lessons

The authors have learned how to diagnose a coronary fistula, manage its complications and treatment and searched the literature about its frequency and treatment modalities.

Peer review

Interesting case report of a rare congenital coronary artery anomaly. This case represents the dilemmas of diagnosis, treatment and follow up of this rare cases.

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Case of cannabinoid hyperemesis syndrome with long-term follow-up

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Key words: Cannabinoids; Hyperemesis; Prognosis; Abdominal pain; Adverse drug effect

Core tip: Cannabinoid hyperemesis syndrome (CHS) can be diagnosed with characteristic clinical features, including long-term cannabis use, severe cyclical abdominal pain, nausea and vomiting, and temporary relief of symptoms with hot showers or baths. Excellent long-term prognosis of CHS can be achieved when abstinence from cannabinoid is maintained. Physicians should have a high index of suspicion in patients with unexplained chronic abdominal pain and vomiting.

Cha JM, Kozarek RA, Lin OS. Case of cannabinoid hyperemesis syndrome with long-term follow-up. *World J Clin Cases* 2014; 2(12): 930-933 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i12/930.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i12.930>

Abstract

Long-term cannabis use may be associated with attacks of severe nausea and vomiting, and a characteristic learned behavior of compulsive hot bathing, termed cannabinoid hyperemesis syndrome (CHS). Long-term follow-up and prognosis of CHS have not been reported previously. A 44-year-old Caucasian man with a long history of addiction to marijuana presented with chronic abdominal pain complicated by attacks of uncontrollable vomiting for 16 years. He had a compulsion to take scalding hot showers, as many as 15 times a day, to relieve his symptoms. All previous therapies had been ineffective. However, abstinence from marijuana led to rapid and complete resolution of all symptoms and his compulsive hot showering behavior. He has been followed for nine years, and is still doing well without recurrence of symptoms. Physicians should have a high index of suspicion for this under-recognized condition, as excellent long-term prognosis of CHS can be achieved when abstinence is maintained.

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INTRODUCTION

According to the World Health Organization, drug abuse remains prevalent around the globe, and about 27 million individuals worldwide are addicts^[1]. Although legal recreational drugs, such as tobacco and alcohol, boast higher rates of consumption, cannabis is the most commonly used illegal recreational drug in the world^[2,3]. In some patients, long-term cannabis use is associated with severe episodes of nausea and vomiting, and a characteristic learned behavior of compulsive hot bathing. So-called cannabinoid hyperemesis syndrome (CHS) was first described in 2004 by Allen *et al*^[4] and its features were confirmed by several other subsequent reports^[5,6]. This condition may be underdiagnosed because of relatively recent recognition and lack of awareness. Physicians should be vigilant for this unique cluster of symptoms to avoid misdiagnosis even after a protracted, invasive and costly workup. Long-term follow-up and prognosis of

CHS has not been previously reported, therefore we now present a case of CHS with follow-up of nine years.

CASE REPORT

A 44-year-old Caucasian man with a long history of marijuana use presented to our clinic with chronic episodic abdominal pain complicated by attacks of uncontrollable vomiting for the past 16 years. His abdominal pain centered in the epigastrium or periumbilical region, occurred abruptly without provocation, was often aggravated by eating in the morning, and lasted anywhere from three hours to two days. Because of his abdominal pain, his weight fluctuated between 50–68 kg. In recent years, all his symptoms had increased in intensity and frequency. He had a compulsion to take scalding hot showers, as many as 15 times a day, to relieve his symptoms. When he ran out of hot water at home, he would drive to his mother's house, his sister's house, or visit his neighbors, even in the middle of night. Four years prior to his presentation to us, he had been hospitalized with second degree burns on his back because of the showers. In the last few years, he had undergone a massive workup from five previous gastroenterologists, and had visited the emergency room more than 20 times. He denied tobacco, alcohol or illegal drug use, with the exception of regular marijuana use for the past 20 years, consuming at least 4–8 marijuana doses ("joints") per day. He had no specific family history, except for Crohn's disease in a cousin.

His previous gastrointestinal workup had been extensive, including numerous abdominal and pelvic computerized tomographs (CTs), at least two small bowel follow-through studies, multiple abdominal ultrasounds, and a barium swallow and head CT. All were negative. Twenty-four hour urine porphyrin levels were normal and urinalysis did not show occult blood. Three years ago, he underwent upper endoscopy and colonoscopy, which were also unremarkable. In the past, treatment attempts had been made with psychotropic and neuromodulatory medications (including amitriptyline, paroxetine hydrochloride, sertraline and tegaserod), dietary manipulation, and alternative medical therapies. However, all of these efforts had been ineffective. This condition adversely affected all aspects of his life, including his relationship with family, friends and peers, making him unemployable. His workup was estimated to have cost tens of thousands of dollars.

He was thin with a weight of 50 kg, and his physical examination was otherwise unremarkable. His laboratory and radiological tests were within normal limits. Blood tests showed no abnormalities, including a liver functional panel, amylase and lipase. Stool occult blood tests were negative. Repeat abdominal ultrasound and abdomen and pelvic CT were normal. A repeat small bowel follow-through and capsule endoscopy were performed to exclude small bowel Crohn's disease, as he had a history of unexplained perirectal fistulae as well as a distant relative with Crohn's disease. The small bowel follow-through was negative, but multiple small ulcerations

scattered throughout the small intestine were noted on capsule endoscopy. However, the mucosa did not appear to be inflamed, and there were no strictures, masses, or signs of bleeding, therefore the small ulcers were considered an incidental finding. The gastric emptying time for the capsule was only three minutes. A urine toxicology screen was done to rule out other recreational drugs, and was negative.

He was asked to stop marijuana use because of the concern for CHS. Abstinence led to a dramatic improvement within one week, with complete resolution of all symptoms and compulsive hot showering behaviors. Since then, he has gained 20 kg, completed a college degree, found employment, gotten married and started a family. We have had 9 years of follow-up so far, and he is still doing well without recurrence of symptoms. He speaks at educational events on the impact of marijuana on his life.

DISCUSSION

Large, population-based surveys suggest that illicit drug use is relatively common in the population, with initial use typically starting in mid to late adolescence: cannabinoids are the most commonly used illegal substances^[7,8]. Cannabinoids have also been used for the treatment of nausea, vomiting, anorexia and anxiety^[5]. Its mechanism of action for inhibiting nausea and vomiting is not precisely known, but is probably related to stimulation of cannabinoid receptors in the brain. Given the nationwide increase in cannabinoid use for recreational and medical reasons, adverse drug effects associated with cannabinoid have become more prominent.

Chronic use of cannabinoids in some individuals can paradoxically cause severe episodic abdominal pain, nausea and vomiting^[4]. Recently, Simonetto proposed clinical criteria for CHS^[6]. Major diagnostic features include long-term cannabis use, severe cyclical abdominal pain, nausea and vomiting, resolution with cannabis cessation, and temporary relief of symptoms with hot showers or baths. The patient in our case report demonstrated all these features; in particular, he indulged in compulsive hot bathing behavior during acute attacks, a phenomenon prominently seen in almost all prior reports in the literature^[9–14]. Supportive diagnostic features include age younger than 50 years, weight loss of greater than 5 kg, morning predominance of symptoms, normal bowel habits and negative findings on diagnostic testing. Our case also showed all the secondary features, with his social and work life severely affected by CHS. In the past, long-term follow-up and prognosis for this condition have not been reported because CHS has only recently been recognized and the recidivism rate is high in patients who are not determined to get better. It should be noted that some patients are psychologically addicted to marijuana and exhibit considerable denial when confronted with the possibility that marijuana, which has purported anti-nausea properties, may be the cause of their chronic nausea and abdominal pain symptoms. Patients who are not determined to get

better may have difficulty maintaining abstinence from marijuana for long periods of time. Our case demonstrates that prolonged abstinence leads to sustainable improvements in all symptoms over a period as long as nine years.

The mechanism of CHS is still unknown. Most cannabinoids act through two receptors, CB1 and CB2, which reduce anterior pituitary hormone and increase corticotrophin release^[15]. Disturbances of the hypothalamic-pituitary-adrenal axis and the presence of autonomic instability have been proposed as possible mechanisms of CHS^[6]. The central effect of long-term cannabis use is thought to be similar to that seen in cyclic vomiting syndrome, which is characterized by the increased secretion and activation of corticotrophin-releasing factor^[16]. In addition, relief of symptoms with compulsive hot bathing might be due to impairment of physiologic thermoregulatory mechanisms by cannabinoids^[6], as CB1 receptors of the preoptic area have been reported to be involved in the hypothermic effects of cannabinoids^[17,18]. As peripheral CB1 receptors in the gastrointestinal tract have also been implicated in slowing gastrointestinal transit^[19], it is suggested that slowed gastric emptying might be responsible for the severe vomiting seen in CHS^[4]. However, only 30% of CHS patients had delayed gastric transit, with the majority having either normal or increased gastric transit on gastric scintigraphy^[6].

The diagnosis of CHS can be made if there is a high index of suspicion; the pathognomonic feature of compulsive bathing is particularly useful because this phenomenon is not seen in any other condition. As diagnosis of CHS is based on only clinical criteria^[6], laboratory or radiological data are not required for its diagnosis except to rule out other gastrointestinal conditions. Although blood or urine cannabinoid metabolites were not measured in our case, they may be helpful in ruling out the use of other recreational drugs. The correct diagnosis can often prevent an extensive and fruitless medical workup and lead to complete resolution of symptoms once abstinence from marijuana is achieved. Therefore, the index of suspicion amongst the medical profession should be raised, as this may be only the tip of the iceberg given the increasing use of marijuana associated with its legalization in several American states^[1].

In conclusion, physicians should have a high index of suspicion in patients with unexplained chronic abdominal pain and vomiting, because an excellent long-term prognosis of CHS can be achieved when abstinence is maintained. Since the mechanism by which cannabis induces hyperemesis is unknown, further research is required in patients with CHS.

COMMENTS

Case characteristics

A 44-year-old man with a history of marijuana use presented with chronic abdominal pain complicated by attacks of uncontrolled vomiting for 16 years.

Differential diagnosis

Cyclic vomiting syndrome or small bowel inflammatory bowel disease.

Laboratory diagnosis

All laboratory findings were unremarkable, including normal 24-h urine porphyrin levels and urinalysis.

Imaging diagnosis

Repeat abdominal ultrasound, abdomen and pelvic computerized tomograph as well as small bowel follow-through were all normal.

Treatment

All previous treatments were ineffective, including psychotropic and neuro-modulatory medications (amitriptyline, paroxetine hydrochloride, sertraline, and tegaserod), dietary manipulation, and alternative medical therapies, however, abstinence of marijuana led to a dramatic improvement of all symptoms within one week.

Related reports

Cannabinoid hyperemesis syndrome (CHS), which is caused by chronic use of cannabis, may be associated with severe episodes of nausea and vomiting, and a characteristic learned behavior of compulsive hot bathing. This condition may be underdiagnosed because of relatively recent recognition and lack of awareness. Physicians should be aware of this unique cluster of symptoms to avoid misdiagnosis even after a protracted, invasive and costly workup.

Term explanation

CHS can be diagnosed based on major clinical features including long-term cannabis use, severe cyclical abdominal pain, nausea and vomiting, resolution with cannabis cessation, and temporary relief of symptoms with hot showers or baths.

Experiences and lessons

This case report highlights the excellent prognosis of CHS when abstinence from cannabis is maintained. Physicians should have a high index of suspicion for this rare condition in patients with unexplained chronic abdominal pain and vomiting. This report provides useful information on a rare disease as the cause of chronic abdominal pain and vomiting.

Peer review

This is an interesting case study of cannabinoid hyperemesis syndrome, which is characterized by chronic, heavy use of cannabis, recurrent episodes of severe nausea and intractable vomiting, and abdominal pain. Overall, the paper is well written.

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Scalp block for brain abscess drainage in a patient with uncorrected tetralogy of Fallot

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INTRODUCTION

Tetralogy of Fallot (TOF), with an incidence of 10% of all congenital heart diseases^[1], is the commonest cyanotic congenital heart disease^[1] and has a dilated aorta which overrides the large ventricular septal defect along with right ventricular outflow tract (RVOT) obstruction and hypertrophy of right ventricle. RVOT can be valvular, infundibular or both^[2]. There have been several case reports of successful management of TOF presenting for brain abscess drainage, cesarean section and major abdominal surgeries^[3-5]. We present a case report describing the use of scalp block combined with sedation for brain abscess drainage in a child with uncorrected TOF.

CASE REPORT

An 11-year-old male child weighing 44 kg presented to us in the emergency department with a history of fever up to 102 °F, headache and vomiting for 10 d. The child was a known case of TOF but had not undergone any surgical repair. His effort tolerance was poor. He had a history of cyanotic spells since childhood but was not on any medication. On examination, the child was conscious, irritable and crying. The child did not show any signs of raised intracranial pressure. Central cyanosis and clubbing were present. He had a pulse rate of 76 per minute with a blood pressure of 110/60 mmHg. On examination of the cardiovascular system, the apex beat was found in the left 5th intercostal space in the midclavicular line and was associated with a left parasternal heave. 1st and 2nd heart sounds and a loud pulmonary component of the 2nd heart sound were audible, along with a pansystolic murmur (Grade 4/6) at the left lower sternal border. No focal deficit was found on neurological examination. The

Abstract

We report a case of an 11-year-old boy with diagnosed but uncorrected tetralogy of Fallot presented to us for brain abscess drainage. The child was managed successfully with scalp block with sedation.

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Key words: Tetralogy of Fallot; Brain abscess; Ketamine; Scalp block; Congenital heart disease

Core tip: We present a case report describing the use of scalp block combined with sedation for brain abscess drainage in a child with uncorrected tetralogy of Fallot. The goal should be to maintain hemodynamic stability and avoid any increase of a right to left shunt. Therefore, we decided to perform scalp block combined with sedation in this child. We used O₂ inhalation, analgesia and sedation with fentanyl, midazolam and ketamine to alleviate anxiety and increase systemic vascular resistance, pulmonary perfusion and oxygenation.

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Figure 1 Chest X-ray of the patient showing enlarged cardiac silhouette.

respiratory and gastrointestinal systems were normal on examination.

Chest X-ray (Figure 1) showed an enlarged cardiac shadow with left ventricular hypertrophy and dilated pulmonary arteries. Electrocardiogram (ECG) revealed sinus rhythm with right ventricular hypertrophy. Echocardiography (ECHO) showed a large ventricular septal defect (VSD) of 13 mm, 60% overriding of the aorta, right ventricular hypertrophy and right ventricular tract outflow obstruction and ejection fraction of 0.6. Cardiac catheterization was not done. Contrast-enhanced computed tomography (Figure 2) showed a left temporo-parietal abscess with no uncus herniation, along with a midline shift of 2 mm. His hematocrit was 58% with a platelet count of $105 \times 10^9/L$. The child's serum electrolytes, coagulation studies and renal function tests were within normal limits. The baseline Arterial Blood Gas analysis revealed pH 7.419, P_{O_2} 35.5 mmHg, P_{CO_2} 31.8 mmHg, HCO_3^- 20.1 mEq/L, SPO_2 68.7% and base excess -3.3. The child received infective endocarditis prophylaxis prior to the surgery, was allowed oral intake of fluids up to 2 h before surgery and normal saline was used as the maintenance fluid thereafter in the ward. In the operation theater, standard monitoring was done with noninvasive blood pressure, pulse oximetry, ECG and temperature. A NeoStar™ triple lumen central venous catheter was in situ as it was inserted when the child presented to us in the emergency department. A 20 G arterial cannula (Becton Dickinson Critical Care Systems, Singapore) was inserted into the radial artery under local anesthesia. The baseline heart rate was 70 beats per minute with an invasive blood pressure of 116/68 mmHg and a central venous pressure (CVP) of 10 cm of H_2O . The child had 64% SPO_2 with 50% O_2 with a Venturi face mask. Normal saline was used as maintenance fluid with the dose of 4 mL/kg per hour.

The scalp block was given with 20 mL of 0.75% ropivacaine without adrenaline (3–4 mL for each nerve) to block the supratrochlear, supraorbital, zygomaticotemporal, auriculotemporal, greater and lesser occipital nerve. The block was supplemented with fentanyl 20 μg *iv*, followed by ketamine 20 mg *iv* and midazolam 0.2 mg *iv* at the time of the burr hole. The child was kept on spontaneous respiration throughout the procedure with a 50% oxygen and air mixture. At the time of dural opening, intra-

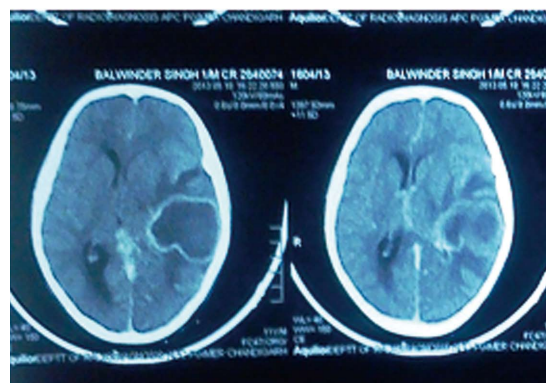


Figure 2 Contrast-enhanced computed tomography of the patient showing left temporo-parietal abscess.

venous ketamine 20 mg was repeated. Normal saline was administered to keep CVP in range of 14–16 cm of H_2O . The procedure lasted for 30 min and the intraoperative course was uneventful, with maintenance of hemodynamic parameters and acid base status within normal limits and a SPO_2 of 69.5%.

DISCUSSION

For the anesthetic management of these patients, one should be careful about the drugs and events that may increase the R-L shunt^[6]. The severity of the disease directly correlates with the size of VSD, severity of pulmonary stenosis and functional status of the right ventricle^[7]. Complications of right to left shunts include chronic hypoxia leading to pulmonary vasoconstriction, altered acid base status, polycythemia, coagulopathy, infective endocarditis and cerebral abscess due to increased risk of paradoxical emboli. The reported incidence of brain abscess in patients with cyanotic heart disease is between 5% and 18.7%^[8].

Anesthetic management of these patients is always a challenge for the anesthetists because of the cardiopulmonary and coagulation abnormalities, dehydration and electrolyte imbalance, along with abscess-induced complications of seizures, meningitis and raised intracranial pressure^[4].

General anesthesia with controlled ventilation has the advantage of better oxygenation but can be associated with the risk of hemodynamic instability, along with compression of pulmonary vessels, impaired gas exchange and acidemia^[9].

Most of the agents used for induction and maintenance of general anesthesia may also lead to myocardial depression, along with reduction of SVR.

The goal should be to maintain hemodynamic stability and avoid the changes that would increase the right to left shunt. Therefore, we decided to perform scalp block combined with sedation in this child.

Factors such as thorough preoperative examination, ECHO, treatment of any chest infections, cardiologist consultation, documentation of preoperative cardiac and neurological status and correction of any coagulopathy were necessary and taken care of in our child.

Prolonged fasting is better avoided in these patients and intake of clear fluids up to two hours prior to the surgery should be allowed. We followed the same guidelines in our patient with normal saline as a maintenance fluid in the ward. Prevention of dehydration is also important as these patients have an increased hematocrit. Patients with a hematocrit $\geq 60\%$ are susceptible to develop coagulopathy and preoperative phlebotomy is beneficial in such cases. Our child had a hematocrit of 58% and preoperative phlebotomy was not performed as it was an emergency procedure, but adequate precautions to prevent dehydration and liberal fluid administration were done to keep the CVP in the range of 14-16 cm of H₂O. Fluid boluses of 20 mL/kg may be required to increase the blood pressure and RV preload^[10].

Air bubbles are also a preventable cause of perioperative morbidity in patients with shunting as air or particulate matter may be shunted directly into the arterial bed^[11,12] and we took measures to prevent this.

We used O₂ inhalation, analgesia and sedation with fentanyl, midazolam and ketamine to alleviate anxiety and increase SVR, pulmonary perfusion and oxygenation. Although O₂ inhalation, fentanyl and midazolam cannot increase SVR, they avoid increasing PVR. Ketamine may increase SVR at some level or more importantly can prevent lowering of SVR and it helped our patient by decreasing the left to right shunt.

Ketamine has also been shown to be better in children with pulmonary hypertension^[13,14] although this is not the cause of cyanosis in these patients but it is the fall in SVR leading to left-right shunt which causes hypoxia. In one study of 18 neonates who had complex cardiac defects, ketamine was used most commonly when intubation was not required for surgery^[15]. Anesthetic agents like sevoflurane, isoflurane and fentanyl/midazolam infusions have no effect on the shunt fraction of children with shunts^[16-18].

Scalp block is a well-established technique for craniotomy, increasingly being used for epilepsy surgery, temporal lobectomy where the excision encroaches on eloquent cortex areas^[19].

Scalp block may be given preoperatively to reduce the hemodynamic response to pin holder application and postoperatively before the emergence to decrease the severity of postoperative pain. They also decrease intra and postoperative opioid requirement^[20,21].

We used 0.75% ropivacaine without adrenaline for administering scalp block as the addition of adrenaline may cause tachycardia which is very dangerous in patients with uncorrected TOF because it may cause infundibular spasm and a cyanotic spell.

Scalp block along with sedation is being used successfully in our institute for patients with chronic subdural hemorrhage. Since the patient we encountered had to undergo an emergency procedure with no time for cardiac catheterization and medical optimization of the patient, we decided to proceed with regional anesthesia with sedation and invasive monitoring in the patient.

The avoidance of general anesthesia due to medical

reasons in selected patients and with the thorough anatomical knowledge of nerve blocks, this underestimated regional technique of scalp block with sedation may be considered as an alternative technique in selective patients with unrepaired TOF and has proved to be an extremely rewarding procedure for the neuroanesthetist whilst offering the best possible outcome for the patient.

COMMENTS

Case characteristics

An 11-year-old male, a known case of tetralogy of Fallot (TOF), presented with fever (up to 102 °F), headache and vomiting for the past 10 d.

Clinical diagnosis

On examination, the child had central cyanosis, clubbing, loud P2 and grade IV pansystolic murmur but there were no signs of raised intracranial pressure and no neurological focal deficit.

Differential diagnosis

A known case of TOF who had not undergone any surgical repair and presented with brain abscess.

Laboratory diagnosis

The patient had a high hematocrit with a normal coagulation profile. The baseline Arterial Blood Gas analysis revealed pH 7.419, Po₂ 35.5 mmHg, Pco₂ 31.8 mmHg, HCO₃ 20.1 mEq/L, SPO₂ 68.7% and BE -3.3.

Imaging diagnosis

Echocardiography revealed a large ventricular septal defect of 13 mm, 60% overriding of aorta, right ventricular tract outflow obstruction and ejection fraction of 0.6, while contrast-enhanced computed tomography (Figure 2) showed a left temporoparietal abscess with no uncal herniation and a midline shift of 2 mm.

Pathological diagnosis

The patient was diagnosed as a brain abscess with uncorrected TOF.

Treatment

Child was not on any medication and presented to us in the emergency department. The child received infective endocarditis prophylaxis prior to the surgery.

Experiences and lessons

Uncorrected TOF presents as a challenge to anesthetists and a thorough knowledge about the physiological and pathological changes occurring with the disease is essential for the safe management of the patient in the perioperative period. Regional anaesthesia should be considered as an alternative to general anesthesia when feasible.

Peer review

A good paper that can be accepted.

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Complete oral rehabilitation in a case with severe dental fluorosis

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plane

Core tip: A novel technique of full occlusal rehabilitation is illustrated here. It is a simple procedure which adheres to all the principles of occlusal rehabilitation. Rehabilitation of dental fluorosis using the treatment protocol suggested here will systematize and streamline the clinical technique and it is hoped that this approach will benefit the patients and act as a guide for dentists. Although the technique described here is skill sensitive, it is the author's belief that it is a new paradigm in full mouth occlusal rehabilitation.

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Abstract

The authors have presented a technique of full occlusal rehabilitation in a case of severe dental fluorosis. In this technique, maxillary and mandibular anterior teeth were simultaneously prepared and restored first. This was followed by simultaneous preparation of maxillary and mandibular posterior teeth that were restored in canine guided occlusion. The technique and sequence followed here is unique and is not available in dental literature. This technique reduces number of appointments while fulfilling all objectives. Periodontal follow-up over 3 years was satisfactory. A restorative treatment protocol has been devised for fluorosis which will act as a guide for the dental practitioners.

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Key words: Occlusal rehabilitation; Dental fluorosis; Treatment protocol; Restorative management; Occlusal

INTRODUCTION

There are hardly any documented cases in dental literature where dental fluorosis is treated by full occlusal rehabilitation. Restoration of dentitions affected by dental fluorosis is a challenging prospect. The presence of dental hypoplasia, the severity of discoloration, associated symptoms like hypersensitivity and attrition makes treatment planning extremely critical. The majority of fluorosis patients usually have mild to moderate fluorosis^[1,2] wherein the main symptoms are discoloration and/or very mild hypoplasia^[3,4]. These may be managed by simple restorative procedures like bleaching or composite filling. Some patients have moderate fluorosis which requires veneers or an occasional crown^[5-8]. Very rarely, patients with severe fluorosis require full occlusal rehabilitation. The protocol for treatment of dental fluorosis has been formulated in this article.



Figure 1 Baseline pre-treatment intraoral photograph.

This article documents a case of severe dental fluorosis with intraoral findings such as severe attrition, anterior open bite and unilateral cross bite, which was treated by full occlusal rehabilitation. Novel clinical and technical modifications were employed which may help to simplify the procedure of full occlusal rehabilitation.

CASE REPORT

A 28-year male patient reported to the Department of Prosthodontics, with a chief complaint of inability to chew food and discoloration of teeth. A detailed personal history revealed that the patient belonged to one of the fluoride belts of India. Clinical findings included protruded mandible, concave facial profile, severe dental fluorosis (Level 4 on Dean's Modified Index)^[9], maxillary midline not coinciding with mandibular midline, anterior open bite, unilateral crossbite with a few centric stops on left side, generalized severe attrition of teeth with moderate sensitivity (Figure 1). However, there was no loss of vertical dimension which could be attributed to passive eruption to compensate for the attrition. Diagnostic impressions were made using irreversible hydrocolloid (Zelgan 2002, Dentsply, India) and casts poured using dental stone (Kalabhai Karson Pvt. Ltd., India). Face bow (Hanau Springbow, Waterpik Technologies, United States) transfer was done and the casts were mounted using a centric relation record on a semi-adjustable articulator (Hanau H2, Whip Mix Corp, United States). All the clinical findings were confirmed by diagnostic mounting of the casts. Diagnostic wax up was done based on findings of clinical examination, diagnostic mounting and diagnostic wax up. Full occlusal rehabilitation using ceramometal crowns [Meta Cast (V), United States], without changing the vertical dimension at occlusion, was decided as the treatment of choice. The limitations of this treatment option viz., inability to coincide the maxillary and mandibular midline, inability to improve the facial profile and persistence of crossbite on left side were explained to the patient and his approval was obtained for the treatment plan. Maxillary and mandibular anterior teeth were prepared simultaneously to receive individual ceramometal crowns. Impressions were made using Vinyl Polysiloxane (GC America Inc, Made in Japan) by the putty-

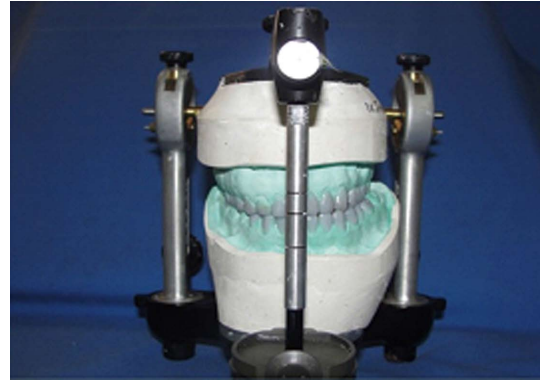


Figure 2 Centric relation and centric occlusion was verified in a semi adjustable Hanau articulator.

wash technique^[10]. Individual temporary crowns (DPI, India) were fabricated using the indirect technique^[11] and were used to establish the anterior guidance in the patient's mouth in such a way that anterior temporaries provided canine guided occlusion. This was transferred to the semi-adjustable articulator and a custom incisal table was fabricated. The anterior metal try-in was carried out and ceramic (Vita VMK 95, Germany) build up was done according to the anterior guidance obtained from the patient. These definitive anterior restorations were seated in the patient's mouth, the canine guided occlusion verified and finally the anterior individual crowns were cemented using glass ionomer luting cement (Ketac Cem, 3M ESPE, Germany). The maxillary and mandibular midlines got close but could not be coincided. In the next phase of treatment, all the posterior teeth (maxillary and mandibular) were prepared simultaneously in a single appointment and a centric relation record was obtained. Impressions were made and master casts poured using die stone (Kalrock, Kalabhai Karson Pvt Ltd, India). The casts obtained were mounted on the semi-adjustable articulator. The horizontal and lateral condylar guidances were set arbitrarily at 20° and 15° respectively^[12]. The metal copings were fabricated and tried in the patient. Before the ceramic build up was started, the occlusal plane had to be established. This was set at the midpoint between the prepared maxillary and mandibular posterior teeth. After ceramic build up, the definitive restorations were tried in the patient, harmony of centric relation and centric occlusion was verified (Figure 2), canine guided occlusion was confirmed and the restorations were cemented.

Follow up

Follow up of the restorations and surrounding tissues was done for 3 years. Gingival and Periodontal component of Periodontal Disease Index (PDI)^[13] and Plaque component of PDI (Shick and Ash modification) was recorded at the beginning of treatment, every 3 mo for 1 year post treatment and every 6 mo for next 2 years (Figure 3). The Gingival and Periodontal component of PDI score before treatment was 2, in the first year post treatment it was 1 and for the next two years it was 0. Plaque

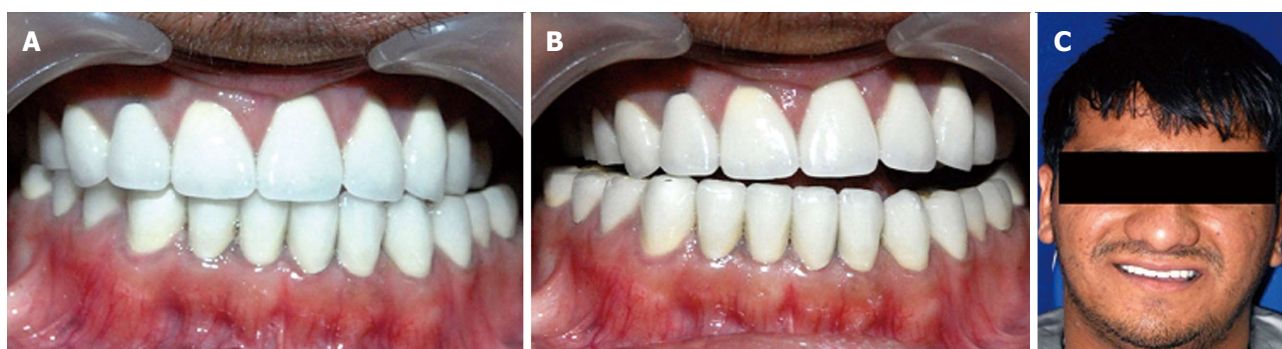


Figure 3 Follow up of the restorations and surrounding tissues. A: One year post-treatment intraoral photograph; B: Two year post-treatment intraoral photograph; C: Three years post-treatment extra oral photograph.

Table 1 Restorative treatment protocol for dental fluorosis of varied severity

Modified dean's fluorosis index score	Clinical findings	Suggested treatment options
Normal (0)	Enamel represents usual transparency, semi-vitriform type of structure. The surface appears smooth, glossy and usually of a pale, creamy white colour	No treatment
Questionable (0.5)	Few flecks to occasional white spots	No treatment/bleaching
Very Mild (1)	Small, opaque, paper white areas scattered over < 25% of the tooth surface	No treatment /bleaching
Mild (2)	White opaque areas in enamel of the teeth are more extensive, but do not involve as much as 50% of the tooth	Bleaching/ composite restoration
Moderate (3)	All enamel surfaces of the teeth are affected and surfaces subject to attrition show wear. Brown stain is frequently a disfiguring feature	If discolorations accompanied by wear: Full coverage If only discoloration without any wear: 1 Bleaching or 2 Veneers (Direct or indirect) or 3 Bleaching followed by Veneers (Direct or indirect)
Severe (4)	All enamel surfaces affected, severe hypoplasia, discrete or confluent pitting. Brown stains are widespread and teeth often present a corroded-like appearance	Full coverage

component of PDI score before treatment was 2 and for the next three years it was 1. This indicated high compliance of oral hygiene instructions post-treatment by the patient and successful integration of the restorations in harmony with the periodontal apparatus. The patient expressed satisfaction with treatment and esthetics and restorations were sound and asymptomatic (no sensitivity to heat or cold, no pain/tenderness) at the follow-up visits. This three year follow up has reinforced that the treatment plan was sound and objectives of full occlusal rehabilitation were fulfilled while addressing all the pre-treatment problems of the patient.

DISCUSSION

Dental fluorosis is seldom so severe^[14-16] as to warrant full occlusal rehabilitation. In addition, complexities such as unilateral cross bite on left side, minimum occlusal contacts on right side, anterior open bite (as found in the present case) makes the prosthetic rehabilitation of such a patient challenging. Every attempt was made in this case to provide the best possible functional and aesthetic rehabilitation of the patient.

The dentition in full occlusal rehabilitation cases are restored variously following different principles and phi-

losophies. The canine guided occlusion is the favoured occlusal scheme, most often adopted in full occlusal rehabilitation^[17-24]. In this technique, the posterior teeth contact only in centric relation, the incisors are the only teeth contacting in protrusion and the canines are the only teeth contacting in mandibular lateral movements. In this patient, the canine guided occlusal scheme was implemented.

In canine guided occlusion the orientation and location of occlusal plane is not critical as long as it allows the anterior guidance to do its job. In this case, the occlusal plane was planned to be located midway between the prepared posterior teeth. This concept was relatively easy to apply as both the maxillary and mandibular posteriors were prepared at the same time and made the technician's job much easier. Since the technician received both maxillary and mandibular final casts with prepared posterior teeth, it was easier for her to establish proper contours and height of opposing restorations making optimum use of the available space. This technique is especially advantageous in cases of full occlusal rehabilitation restored using canine guided occlusion. In the present case, a technique has been attempted which simplifies the clinical and laboratory procedures of full occlusal rehabilitation while fulfilling all its objectives^[12,25].

The restorative procedures were divided into two components: anterior segment restoration followed by posterior segment restoration. The maxillary and mandibular anterior restorations were fabricated at the same time. Establishing the anterior guidance was also easier. Any adjustments and trimming could be done easily. When the posterior restorations were fabricated, developing the occlusal plane was greatly simplified as both the maxillary and mandibular segments were simultaneously prepared. The occlusal level was then set at the midpoint between the prepared maxillary and mandibular posterior teeth on the articulated casts.

Some occlusal rehabilitation philosophies recommend the restoration of posterior teeth prior to that of anterior teeth (*e.g.*, Hobo Twin-Stage procedure^[26] -Conditions 1 and 2). Other philosophies of full occlusal rehabilitation, including the Panky-Mann-Schuyler concept modified by Dawson^[12] recommend the sequential restoration of mandibular anterior segment, maxillary anterior segment, mandibular posterior and finally the maxillary posterior segment. The approach discussed in this article is unlike any other philosophies of full occlusal rehabilitation, is simple, requires least number of appointments, is unique and novel, and yet it fulfils all the requirements of full occlusal rehabilitation.

Hence clinical work is greatly simplified and patient appointments are limited to just 6 as follows: Appointment 1: Diagnostic impression, face bow transfer. Appointment 2: Preparation of maxillary and mandibular anterior teeth, impressions, temporization of anteriors. Appointment 3: Anterior metal try-in. Appointment 4: Cementation of anterior ceramometal crowns, selective grinding, finishing, polishing; preparation of all posterior teeth; impressions, face bow transfer, temporization of all posterior teeth. Appointment 5: Metal try-in of posterior restorations; Appointment 6: Cementation of posterior ceramometal crowns selective grinding, finishing, and polishing. Appointment 4 may be split into two depending on convenience of operator and/or patient.

Depending on the Modified Dean's Fluorosis Index^[9] which is the gold standard for quantifying dental fluorosis, a treatment protocol is herewith suggested (Table 1) which is meant as a guide; the operator may follow any treatment modality given in the protocol depending upon the skill-philosophy-convenience-preference.

The technique of full occlusal rehabilitation illustrated here simplifies the procedures while adhering to all its principles. Rehabilitation of dental fluorosis using the treatment protocol suggested here will systematize and streamline the clinical procedure and it is hoped that this approach will benefit the patient and act as a guideline for dentists.

COMMENTS

Case characteristics

This is a report of a case of severely discolored teeth, secondary to dental fluorosis; with generalized sensitivity, inability to chew food from both sides and deformed esthetics due to anterior open bite.

Clinical diagnosis

The patient had a concave facial profile with severe dental fluorosis (level 4 on Dean's Modified Index), with prognathic mandible, maxillary midline not coinciding with mandibular midline, anterior open bite, unilateral cross bite with a few centric stops on left side, and severe generalized attrition with moderate sensitivity.

Differential diagnosis

The differential diagnosis can be hypoplasia secondary to trauma to the teeth and jaws, any infections during pregnancy or infancy, poor pre-natal and post-natal nutrition, hypoxia, exposure to toxic chemicals and a variety of hereditary disorders, irregular vitamin D metabolism (vitamin D-resistant rickets) or chronic kidney failure at the time of tooth development.

Laboratory diagnosis

The tests included intra oral periapical and extra oral panoramic radiographs, diagnostic model mounting, pulp vitality testing of all teeth that confirmed the clinical findings of permanent hypoplastic teeth with sensitivity that were severely attrited and in malocclusion.

Imaging diagnosis

Imaging techniques used were orthopantomograph and intraoral periapical radiographs which showed generalized hypoplastic teeth, malocclusion, and anterior open bite.

Treatment

The treatment given was a full mouth rehabilitation using a specialized, simplified technique which is novel.

Term explanation

All terms are standard and established which have been used empirically.

Experiences and lessons

The approach to a full mouth rehabilitation case has to be holistic, patient specific and should fulfill all the criteria of scientific treatment protocol.

Peer review

This is an interesting and well written article. Methods are appropriate. Results are clearly presented. Discussion and Conclusions are really interesting.

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Journals

English journal article (list all authors and include the PMID where applicable)

- 1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol*

2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

- 2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarhoea. *Shijie Huaren Xiaobua Zazhi* 1999; **7**: 285-287

In press

- 3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

- 4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

- 5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

- 6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

- 7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

- 8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

- 9 Outreach: Bringing HIV-positive individuals into care. *HRS-A Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

- 11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

- 12 **Breedlove GK**, Schorffheide AM. Adolescent pregnancy. 2nd ed. Wiczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

- 13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

- 14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

- 15 Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis* serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/>

ncidod/eid/index.htm

Patent (list all authors)

- 16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean \pm SD or mean \pm SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as *v* (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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Use SI units. For example: body mass, *m* (B) = 78 kg; blood pressure, *p* (B) = 16.2/12.3 kPa; incubation time, *t* (incubation) = 96 h, blood glucose concentration, *c* (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, *p* (CEA) = 8.6 $24.5 \mu\text{g/L}$; CO₂ volume fraction, 50 mL/L CO₂, not 5% CO₂; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

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Italics

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