

# World Journal of *Clinical Cases*

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## Minimizing right ventricular pacing in sinus node disease: Sometimes the cure is worse than the disease

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features of these algorithms may lead to adverse (often under-appreciated) consequences in some patients. We describe a case of a patient with sinus node disease, in whom right atrial only pacing involved long atrio-ventricular delay to allow intrinsic ventricular conduction, which led to symptomatic hypotension that could be overcome only by "forcing" also right ventricular apical pacing. We subsequently discuss this case in the context of current available literature.

**Key words:** Right ventricular apical pacing; Pacemaker algorithms; Dyssynchrony; Pacemaker syndrome; Right atrial pacing

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**Core tip:** Right ventricular apical pacing has been associated with worse outcome so a series of pacing algorithms have been designed to minimize it. However the operational features of these algorithms may lead to adverse consequences in some patients. We describe a case of a patient with sinus node disease, in whom right atrial only pacing involved long atrio-ventricular delay to allow intrinsic ventricular conduction, which led to symptomatic hypotension that could be overcome only by "forcing" right ventricular apical pacing. We subsequently discuss this case in the context of current available literature.

### Abstract

Traditional right ventricular (RV) apical pacing has been associated with heart failure, atrial fibrillation and increased mortality. To avoid the negative consequences of RV apical pacing different strategies have been developed, among these a series of pacing algorithms designed to minimize RV pacing. These functions are particularly useful when there is not the need for continuous RV pacing: intermittent atrio-ventricular blocks and, mainly, sinus node disease. However, in order to avoid RV pacing, the operational

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### INTRODUCTION

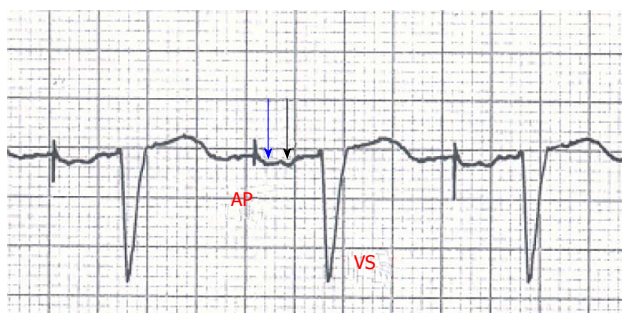
Traditional right ventricular (RV) apical pacing has been associated with heart failure, atrial fibrillation

and increased mortality<sup>[1]</sup>. Dyssynchronous electrical activation of the heart from pacing creates deleterious myocardial fiber strain, mechanically inefficient contraction and adverse left ventricular (LV) remodeling with a progressive, dose-related, decline in pump function that is more evident in patients with an already compromised cardiac function at baseline<sup>[2]</sup>. To avoid the negative consequences of RV apical pacing different strategies have been developed. Pacing from alternative RV sites (septum, outflow tract, His bundle) is a promising option but, up to now, studies comparing apical to non-apical pacing with regard to hemodynamic, echocardiographic and long-term LV systolic function have been conflicting, failing to demonstrate a clear benefit<sup>[3,4]</sup>. Biventricular pacing can be considered in selected cases, *i.e.*, patients with atrio-ventricular (AV) block and LV ejection fraction < 35%, but it is not a first choice in the majority of patients candidates to receive a pacemaker. Contemporary pacemakers, from different manufacturers, include sophisticated algorithms designed to minimize ventricular pacing with the aim to reduce the incidence of atrial fibrillation and heart failure<sup>[5]</sup>. These functions are particularly useful when there is not the need for continuous RV pacing, that is in patients with intermittent AV blocks and, mainly, sinus node disease (SND). However, in order to avoid RV pacing, the operational features of these algorithms involve long AV delay to allow intrinsic conduction, which may lead to adverse (often under-appreciated) consequences in some patients<sup>[5,6]</sup>.

We describe the case of a 85-year-old man implanted with a dual chamber pacemaker (Adapta DR Medtronic, leads positioned in right atrial appendage and RV apex) because of sinus node disease/bradi-tachi syndrome. He did not take any drug. Baseline ECG showed sinus bradycardia (35-40 beats per minute), normal P wave morphology, PR interval 180 msec, QRS 115 msec. Echocardiogram revealed moderate LV hypertrophy, ejection fraction 50%; diastolic pattern (pulsed wave mitral Doppler) showed abnormal relaxation with an adequate filling time in sinus rhythm. Before pacemaker implant he was symptomatic for palpitations, easy fatiguability, dizziness, vertigo, but no syncopal episode was described. Pacemaker was programmed DDDR 60-120 bpm, Managed Ventricular Pacing (MVP)<sup>TM</sup> turned ON to avoid unnecessary RV pacing; ECG after implant showed atrial-based pacing with spontaneous ventricular activation (AP-VS) (Figure 1). Few days after hospital discharge the patient returned to our attention for episodes of near-syncope and falls occurring shortly after the passage from supine to upright position. We documented a symptomatic orthostatic hypotension with a sudden drop in systolic and diastolic blood pressure (SBP drop 30-40 mmHg and DBP drop 15-20 mmHg); during the episodes heart rate increased to about 80-90 beats per minute due to sensor-driven atrial pacing (with spontaneous

ventricular activation). There was no obvious cause to justify these episodes, in particular different etiologies of orthostatic hypotension (neurogenic, non-neurogenic, drug/toxins effect) had been excluded; pacemaker did not show any malfunction. So we repeated an echocardiogram, recording diastolic filling during the episodes of symptomatic orthostatic hypotension: we found E/A wave fusion with a particularly short diastolic filling time (about 280 msec at 80 beats per minute) (Figure 2A). We also noticed that at ECG, during atrial-based pacing, AP-VS interval was about 280-300 msec and right atrial stimulus artifact was followed by a first deflection corresponding to right atrial depolarization (white arrow in Figure 1) and then a second deflection corresponding to left atrial depolarization (black arrow in Figure 1). We also tried to program the pacemaker in AAI mode with a fixed rate but the results were the same compared to MVP<sup>TM</sup>. All these features suggested us that atrial-based pacing was responsible of an abnormal prolongation of AV interval (with E/A fusion), likely associated with intraatrial and interatrial conduction delay, with symptoms (near-syncope) and orthostatic exacerbation similar to "pacemaker syndrome". However, by definition pacemaker syndrome occurs when there is atrial systole during ventricular systole while E/A fusion seen in our case is a diastolic filling issue. So MVP<sup>TM</sup> was turned OFF and we optimized the programmed AV interval to 90 msec ("forcing" RV pacing) in order to ensure an adequate echocardiographic diastolic filling time, with a good separation of E and A waves at pulsed wave mitral Doppler (diastolic filling time 547 msec at 70 beats per minute) (Figure 2B). Since the first day after reprogramming the device, the episodes of orthostatic hypotension did not occur anymore; we tested the patient with an "orthostatic stress test" (a sudden change from supine to upright position while monitoring blood pressure, heart rate and diastolic filling pattern) and neither hypotension nor symptoms occurred during AP-VP paced rhythm.

Diastole begins soon after the end of systolic ejection (aortic valve closure) and includes LV pressure fall, rapid filling, diastasis and atrial contraction. Diastolic filling and cardiac output are strictly linked and the optimal performance of the LV depends on the alternation between a compliant chamber in diastole (LV filling from a low atrial pressure) and a stiff chamber in systole (ejection of the stroke volume at arterial pressures). When passing from supine to upright position there is a venous pooling in the lower extremities and splanchnic circulation as a result of the gravitational change. The consequent decrease of venous return to the heart leads to a transient reduction of ventricular filling, cardiac output and blood pressure. As compensatory mechanisms sympathetic tone increases and parasympathetic activity decreases: venous return, heart rate and vascular resistance they all increase with the aim of maintaining cardiac

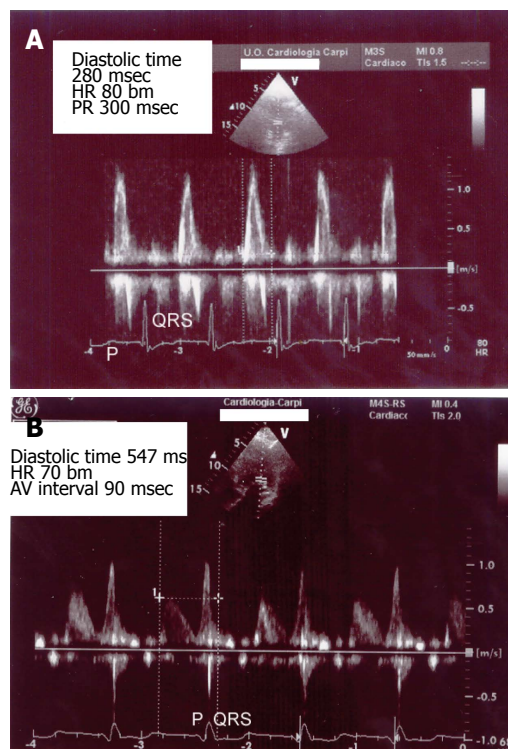


**Figure 1** ECG after implant showing atrial-based pacing with spontaneous ventricular activation. AP-VS interval: 280-300 msec. Right atrial stimulus artifact is followed by a first deflection corresponding to right atrial depolarization (blue arrow) and then a second corresponding to left atrial depolarization (black arrow). AP: Atrial pacing; VS: Ventricular sensing.

output and blood pressure. When one or more of these compensatory mechanisms fail orthostatic hypotension can occur.

Several factors contribute to the clinical, ECG and echo findings in our patient. First of all pacing from right atrial appendage led to a delay in interatrial and intraatrial conduction as manifested by a “wide” P wave following stimulus artifact, with two distinct deflections corresponding to right and left atrial depolarization (Figure 1), while P wave morphology in spontaneous sinus rhythm was completely normal. As a consequence, a long AV delay occurred (AP-VS 300 msec) that was likely and mainly the consequence of inter/intra atrial conduction delay (rather than a true nodal/hisian delay, PR interval being normal during non-paced rhythm). This kind of “pseudo first degree AV block” pushed the A wave toward E wave: E/A fusion occurred so diastolic filling time was abnormally short. During orthostatic challenge the inability to fill the ventricle, because of this diastolic impairment, finally led to symptomatic hypotension with near-syncope. The only way to restore an adequate filling time was to optimize AV delay, but this involved to “force” RV pacing.

MVP™ provides an atrial-based pacing (AAI/R) with ventricular backup at an AV delay of 80 msec in absence of a ventricular sensed event following an atrial sensed or paced event. When loss of AV conduction persists (two out of four non-refractory AA intervals without ventricular sensed events) the pacemaker switches to DDD/R mode at the programmed lower rate and AV delay. The algorithm, then, performs regular checks of AV conduction and switches back to AAI/R mode if possible<sup>[5]</sup>. The MVP™ tolerates markedly prolonged AV delay which can adversely affect cardiovascular hemodynamics, reducing atrial contribution to ventricular filling and favoring diastolic mitral regurgitation<sup>[5,6]</sup>. In general algorithms designed to minimize ventricular pacing operate by prolonging the AV interval with hysteresis or by switching between DDD and AAI modes; the operative features differ between manufacturers but all of them carry the risk



**Figure 2** Pulsed wave mitral Doppler recording. A: Pulsed wave mitral Doppler recording during an episode of symptomatic orthostatic hypotension with atrial-based pacing: E/A wave fusion with a particularly short diastolic filling time (280 msec at 80 beats per minute); B: Diastolic filling after AV delay optimization at 90 msec (“forcing” RV pacing): Good separation of E and A waves at pulsed wave mitral Doppler (filling time 547 msec at 70 beats per minute).

of AV decoupling (defined as > 40% of AV intervals over 300 msec) even when baseline PR interval is normal. To prevent this adverse effect some manufactures have incorporated in their algorithms a maximum tolerated AV delay (350 msec in Ventricular Intrinsic Preference™ by St Jude Medical; 350 msec atrial sensed and 450 msec atrial paced in AAISafeR2™ by Sorin Group): if AV delay exceeds these limits, the device switches to DDD mode.

Atrial pacing “*per se*” increases AV delay: pacing from right atrial appendage can provoke marked alterations in interatrial and intraatrial impulse propagation that impairs coordinated activation and can also favor atrial fibrillation<sup>[7]</sup>. In the DANPACE trial<sup>[8]</sup>, that compared AAI and DDD pacing in SND, atrial-based pacing significantly increased the risk of paroxysmal atrial fibrillation [28.4% in AAI group vs 23% in DDD group; hazard ratio (HR) 1.27;  $P = 0.024$ ]. In a study AAI-R based pacing, in patients with SND and normal baseline PR interval, induced a clinically significant lengthening of AV conduction time, with a paradoxical increase of AV conduction during exercise in 66% of cases (that was predicted by use of antiarrhythmic class I c/III drugs)<sup>[9]</sup>. Moreover in 23%-58% of SND patients AV conduction is already impaired at baseline, two thirds of these patients having a first degree AV block; the optimal pacing mode in these subgroup is not determined. In a



comparison study between conventional dual chamber pacing and minimal ventricular pacing mode there was no significant difference in terms of functional capacity assessed by cardiopulmonary test, quality of life and echocardiographic parameters of systolic/diastolic function; it was concluded that sequential AV pacing may be a reasonable choice for patients with SND and prolonged PR interval<sup>[10]</sup>.

Alternative atrial pacing sites have also been studied: high and low interatrial septum, Bachmann bundle, lateral free wall and combinations of these sites; the concept was to improve atrial hemodynamics by reducing total atrial activation time. Although several small studies indicated that some alternative sites could help to prevent atrial fibrillation, randomized trials did not show benefit in the long term<sup>[11]</sup>.

The attempt to minimize RV pacing, at expense of AV synchrony, can be particularly deleterious in patient with heart failure. In the INTRINSIC RV trial<sup>[12]</sup> patients indicated for ICD implant were randomized to dual chamber pacing with AV Search Hysteresis™ or single chamber VVI pacing 40 bpm. Patients with 10%-19% RV pacing had the most favorable outcome, while the risk of clinical events (mainly heart failure decompensation) in the 0%-9% RV pacing group was as high as the 40%-49% RV pacing group. So some ventricular pacing may be necessary, even if the optimal balance between AV synchrony and intraventricular dyssynchrony induced by RV pacing varies between patients and is not simple to define.

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This statement is to certify that all authors have seen and approved the manuscript being submitted, have contributed significantly to the work, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to the Journal. We attest that the article is the Authors' original work, has not received prior publication and is not under consideration for publication elsewhere. On behalf of all co-authors, the corresponding Author shall bear full responsibility for the submission. Any changes to the list of authors, including changes in order, additions or removals will require the submission of a new author agreement form approved and signed by all the original and added submitting authors. Patient's consent was obtained. The authors report no relationships that could be construed as a conflict of interest.

## REFERENCES

- 1 **Sweeney MO**, Hellkamp AS, Ellenbogen KA, Greenspon AJ, Freedman RA, Lee KL, Lamas GA. Adverse effect of ventricular pacing on heart failure and atrial fibrillation among patients with normal baseline QRS duration in a clinical trial of pacemaker therapy for sinus node dysfunction. *Circulation* 2003; **107**: 2932-2937 [PMID: 12782566]
- 2 **Prinzen FW**, Augustijn CH, Arts T, Allessie MA, Reneman RS. Redistribution of myocardial fiber strain and blood flow by asynchronous activation. *Am J Physiol* 1990; **259**: H300-H308 [PMID: 2386214]
- 3 **Cano O**, Osca J, Sancho-Tello MJ, Sánchez JM, Ortiz V, Castro JE, Salvador A, Olague J. Comparison of effectiveness of right ventricular septal pacing versus right ventricular apical pacing. *Am J Cardiol* 2010; **105**: 1426-1432 [PMID: 20451689 DOI: 10.1016/j.amjcard.2010.01.004]
- 4 **Shimony A**, Eisenberg MJ, Filion KB, Amit G. Beneficial effects of right ventricular non-apical vs. apical pacing: a systematic review and meta-analysis of randomized-controlled trials. *Europace* 2012; **14**: 81-91 [PMID: 21798880 DOI: 10.1093/europace/eur240]
- 5 **Lim HS**. The prescription of minimal ventricular pacing. *Pacing Clin Electrophysiol* 2012; **35**: 1528-1536 [PMID: 22897410 DOI: 10.1111/j.1540-8159.2012.03490.x]
- 6 **Rosenblum AM**. Marked interatrial and atrioventricular conduction delay with enhanced atrial-based managed ventricular pacing: electrocardiogram-echocardiogram Doppler correlation. *Circulation* 2010; **122**: e494-e496 [PMID: 20975005 DOI: 10.1161/CIRCULATIONAHA.110.967240]
- 7 **Vardas PE**, Simantirakis EN, Kanoupakis EM. New developments in cardiac pacemakers. *Circulation* 2013; **127**: 2343-2350 [PMID: 23753845 DOI: 10.1161/CIRCULATIONAHA.112.000086]
- 8 **Nielsen JC**, Thomsen PE, Højberg S, Møller M, Vesterlund T, Dalsgaard D, Mortensen LS, Nielsen T, Asklund M, Friis EV, Christensen PD, Simonsen EH, Eriksen UH, Jensen GV, Svendsen JH, Toff WD, Healey JS, Andersen HR. A comparison of single-lead atrial pacing with dual-chamber pacing in sick sinus syndrome. *Eur Heart J* 2011; **32**: 686-696 [PMID: 21300730 DOI: 10.1093/eurheartj/ehr022]
- 9 **Mabo P**, Cebron JP, Solnon A, Tassin A, Graindorge L, Gras D. Non-physiological increase of AV conduction time in sinus disease patients programmed in AAIR-based pacing mode. *J Interv Card Electrophysiol* 2012; **35**: 219-226 [PMID: 22836479 DOI: 10.1007/s10840-012-9703-4]
- 10 **Krzyżanowski K**, Michałkiewicz D, Orski Z, Wierzbowski R, Ryczek R, Cwetsch A. Minimizing right ventricular pacing in patients with sinus node disease and prolonged PQ interval: the impact on exercise capacity. *Cardiol J* 2014 May 20; Epub ahead of print [PMID: 24846513 DOI: 10.5603/CJ.a2014.0035]
- 11 **Verlato R**, Botto GL, Massa R, Amellone C, Perucca A, Bongiorno MG, Bertaglia E, Ziacchi V, Piacenti M, Del Rosso A, Russo G, Baccillieri MS, Turrini P, Corbucci G. Efficacy of low interatrial septum and right atrial appendage pacing for prevention of permanent atrial fibrillation in patients with sinus node disease: results from the electrophysiology-guided pacing site selection (EPASS) study. *Circ Arrhythm Electrophysiol* 2011; **4**: 844-850 [PMID: 21946316 DOI: 10.1161/CIRCEP.110.957126]
- 12 **Olshansky B**, Day JD, Lerew DR, Brown S, Stolen KQ. Eliminating right ventricular pacing may not be best for patients requiring implantable cardioverter-defibrillators. *Heart Rhythm* 2007; **4**: 886-891 [PMID: 17599672]

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## Current status and emerging challenges in the treatment of hepatitis C virus genotypes 4 to 6

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### Abstract

Hepatitis C virus (HCV) genotypes 4, 5 and 6 are mainly present in Africa, the Middle East and Asia and they have been less extensively studied with respect to epidemiology, natural disease history and therapeutic endpoints. Response rates to a 48-wk combined peginterferon/ribavirin treatment range to 40%-69% for HCV 4, 55%-60% for HCV 5 and 60%-90% for HCV 6. Response-guided schedules are recommended to optimize the outcomes of peginterferon/ribavirin treatment in HCV 4 and, in form of preliminary

data, for HCV 6, but no data are yet available to support such an individualization of therapy for HCV 5. Recently, the direct-acting antivirals (DAAs) with pan-genotypic activities simeprevir, sofosbuvir and daclatasvir have been recommended in triple regimens with peginterferon/ribavirin for the treatment of HCV genotypes 4 to 6 infections. In the future, DAA-based interferon-free therapies are awaited to drastically improve treatment outcomes in HCV. However, efforts to improve treatment outcomes with peginterferon/ribavirin should continue, as the HCV 4-6 infected population is mainly based in resource-limited settings with restricted access to the costly DAAs.

**Key words:** Hepatitis C virus; Genotype 4; Genotype 5; Genotype 6; Pegylated interferon; Ribavirin; Direct-acting antivirals

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**Core tip:** Hepatitis C virus (HCV) 4, 5 and 6 are lesser known genotypes mainly encountered in Africa, the Middle East and Asia. Studies, mostly retrospective, have reported response rates to a 48-wk peginterferon/ribavirin combination ranging to 40%-69% for HCV-4, 55%-60% for HCV-5 and 60%-90% for HCV-6. Increasing evidence has supported a response-guided approach for HCV-4, whereas no robust data are yet available concerning tailoring of treatment duration for HCV-5 and HCV-6. Direct-acting antivirals may significantly improve treatment outcomes in HCV, but use of these agents in countries endemic for HCV 4-6 is currently precluded by the very high costs.

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## INTRODUCTION

Hepatitis C virus (HCV) remains a major health problem worldwide with over 170 million persons chronically infected and a burden of 300000 deaths annually<sup>[1,2]</sup>. Phylogenetic analyses of viral genomic sequences have identified at least 6 major HCV genotypes (and more than 70 subtypes), each with a distinct geographical distribution and sensitivity to antiviral treatment<sup>[3,4]</sup>. HCV 1, 2 and 3 are widely disseminated genotypes and have been thoroughly assessed with regard to epidemiology, natural disease history and treatment outcomes. Conversely, HCV 4, 5 and 6 have a restricted geographical distribution, mainly based in countries with limited resources and research facilities, thus epidemiological reports and treatment advances for these genotypes have been generally deficient. Prevalence of HCV genotypes 4 to 6 across different countries is summarized in Table 1. Genotype 4 is encountered throughout Middle East and Africa<sup>[5-12]</sup>, whereas a spread of the infection has been described in other countries<sup>[13-15]</sup>, particularly in Southern Europe<sup>[16-20]</sup>. HCV 5 is rare outside South Africa<sup>[21-23]</sup>, but its sporadic presence has been reported in different parts of the world<sup>[11,15,24-28]</sup>, including a pocket of the infection in Southeast Greece<sup>[29]</sup>. Lastly, HCV 6 and its subtypes are found mainly in Asia<sup>[30-35]</sup>. Crucially, due to the phenomenon of globalization, the prevalence of the HCV 4 to 6 genotypes outside of these “typical” areas is awaited to increase in forthcoming years.

During the past decade, a dual combination of pegylated interferon (PegIFN) and ribavirin (RBV) has represented the standard of care (SOC) for treating chronic hepatitis C (CHC). In 2011, introduction of first generation direct-acting antivirals (DAAs), the NS3/4A protease inhibitors (PIs) boceprevir and telaprevir, has boosted rates of sustained viral response (SVR; *i.e.*, negative HCV-RNA at 6 mo or more after cessation of treatment) in both naïve and treatment-experienced patients, although this only regarded the most difficult-to-treat CHC genotype 1<sup>[36]</sup>. Latter, in 2013, approval of the second generation DAA, NS5B polymerase inhibitor (PI) sofosbuvir, has been a further step forward due to its pangenotypic effect on HCV, better pharmacokinetics and improved resistance profiles<sup>[37]</sup>. In the light of the rapidly changing paradigm of treating CHC, new drugs were recently approved or await approval. However, in the era of DAAs, optimal treatment of HCV genotypes 4 to 6 remains, more than ever before, to be defined. Indeed, most treatment data rely on retrospective studies, extrapolations using other HCV genotypes as reference, and expert opinions.

Herein, we aimed to a concise overview on the treatment of HCV 4 to 6, including recent proposals for a response-guided treatment approach as well as the available data and future perspectives on the use of DAAs with respect to these lesser known HCV

genotypes.

## TREATMENT OF HEPATITIS C

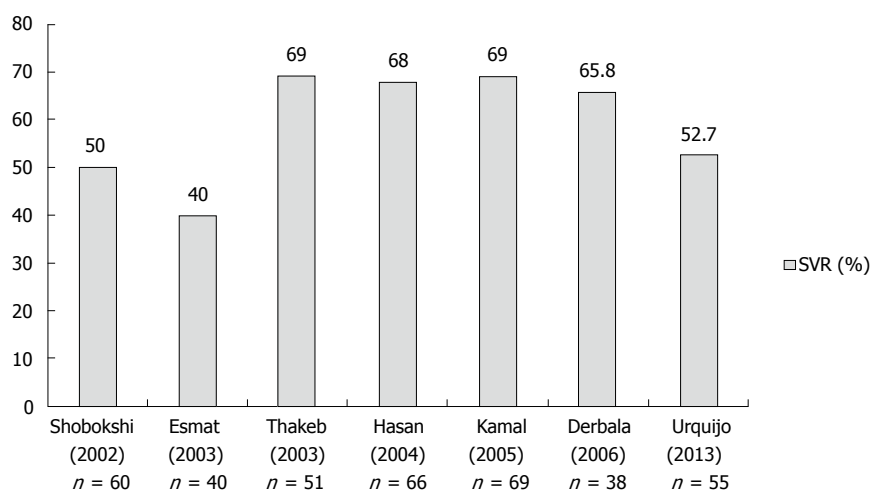
### GENOTYPES 4 TO 6

#### *Combination therapy with PegIFN and RBV*

**Hepatitis C virus genotype 4:** HCV 4 has been traditionally considered a difficult to treat genotype, mainly because of the disappointing SVR rates (5%-25%) obtained in the early clinical trials using conventional interferon monotherapy<sup>[38,39]</sup>. Later introduction of RBV, used in conjunction with PegIFN, has significantly increased the efficacy of treatment, although response rates were still lower as compared to genotype 2 and 3 patients. Figure 1 summarizes results of prospective studies evaluating a fixed 48-wk treatment using standard-dose PEGIFN and RBV (PegIFN $\alpha$ -2a 180  $\mu$ g or PegIFN $\alpha$ -2b 1.5 mg/kg and RBV 1-1.2 g/d) in HCV 4<sup>[40-46]</sup>. Overall, SVR rates ranged between 40% and 69%. However, a significant discrepancy could be noted between the SVR rates reported in highly endemic countries (SVR 60%-69% in studies conducted in Egypt and the Middle East)<sup>[40,42,43,47-50]</sup> and those (generally < 60%) reported in European populations infected with HCV 4; including 55% in Spain<sup>[51]</sup>, 40.3% in France<sup>[52]</sup> and 43.5% in a cohort from Greece<sup>[53]</sup>. This difference has prompted the hypothesis of an impact of ethnicity on antiviral response, with 2 French analyses suggesting Egyptian (vs European) origin as a favorable prognostic indicator for SVR<sup>[52,54]</sup>. However, no solid pathogenetic basis has been provided for this phenomenon, although genetic or immunological ethnic-specific differences have been put forward<sup>[55,56]</sup>. Unlike the French observations, we could not identify any influence of Greek ( $n = 101$ ) vs Egyptian ( $n = 76$ ) origin on treatment outcomes<sup>[57]</sup>, and only age  $\geq 45$  years [odds ratio (OR) = 0.42,  $P = 0.01$ ], presence of diabetes (OR = 0.23,  $P = 0.007$ ), advanced liver fibrosis (Metavir F3-F4; OR = 0.39,  $P = 0.01$ ) and treatment suspension (OR = 0.17,  $P = 0.007$ ) were independent negative associations, in line with previous studies assessing predictors of response in HCV 4<sup>[40,42,43,52,54,58-63]</sup> (Table 2). The importance of metabolic factors has been highlighted by the observation of a beneficial effect of using an insulin-sensitizing agent, such as pioglitazone, in conjunction with antiviral treatment in patients with insulin resistance (homeostasis model assessment index > 2)<sup>[64]</sup>. Congruently, presence of hepatic steatosis, known to be a poor predictor of treatment in CHC, has been linked to host metabolic factors in HCV 4 rather than to a direct viral steatogenic effect as in the case of HCV 3 infection<sup>[65,66]</sup>. Other host factors, including the IL-28B TT genotype and high values of the interferon- $\gamma$  inducible protein 10 (IP-10) have been associated with a poor therapeutic outcome<sup>[59]</sup>. Interestingly, Boglione *et al.*<sup>[67]</sup> have recently proposed use of the IL-28B polymorphisms upstream as a genuine basis for the identification of patients

**Table 1** Indicative reports of hepatitis C virus genotype 4 to 6 prevalence in different continental areas

	Genotype 4		Genotype 5		Genotype 6	
	Country	Prevalence	Country	Prevalence	Country	Prevalence
Africa	Egypt <sup>[10]</sup>	91%	South Africa <sup>[22,23]</sup>	40%		
	Gabon <sup>[12]</sup>	71%				
	Cameroon <sup>[7]</sup>	76%				
	Nigeria <sup>[8]</sup>	60%				
Middle East	Saudi Arabia <sup>[11]</sup>	60%	Syria <sup>[25]</sup>	10%		
	Lebanon <sup>[9]</sup>	30%	Saudi Arabia <sup>[11]</sup>	1%		
	Syria <sup>[5]</sup>	30%				
	Iraq <sup>[6]</sup>	35.40%				
Asia	China <sup>[33]</sup>	0-1.7%			Hong Kong <sup>[33-35]</sup>	10%-30%
					Vietnam <sup>[30]</sup>	14%
					South Korea <sup>[31]</sup>	1.40%
					China <sup>[32,35]</sup>	0-50%
Europe	France <sup>[15]</sup>	4%-10%	France <sup>[15,26]</sup>	3%-14.2%		
	Spain <sup>[17,20]</sup>	1.4%-14%	Belgium <sup>[28]</sup>	1%-5%		
	Italy <sup>[16,19]</sup>	1.4%-3.1%	Spain <sup>[27]</sup>	0-10.3%		
	Greece <sup>[18]</sup>	13.2%-15.2%	Italy <sup>[24]</sup>	0-0.1%		
			Greece <sup>[18,29]</sup>	0.4%-1.9%		
America	United States <sup>[13,14]</sup>	0-2%				

**Figure 1** Prospective studies evaluating a fixed 48-wk treatment using a standard dose of pegylated interferon and ribavirin in patients with hepatitis C genotype 4. SVR: Sustained viral response.**Table 2** Predictors of response to antiviral treatment in patients with hepatitis C virus genotype 4 infection

Predictor	Ref.
Age	[57]
Liver histopathology (advanced fibrosis/severe steatosis)	[40,52,54,60]
Baseline viral load <sup>1</sup>	[42,43]
Ethnicity	[52,54]
Diabetes/Insulin resistance	[49,54,57]
IL28B polymorphisms	[58,63]
Plasma levels of IP-10 <sup>2</sup>	[59]
HCV 4 subtypes	[52]
Co-infections (HIV, Schistosomiasis)	[38,61]

<sup>1</sup>Most studies used a cut-off value of 400,000 IU/mL; <sup>2</sup>Interferon-c inducible protein 10. HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; IP-10: Inducible protein 10.

unlikely to respond to standard dual therapy, and thus candidates for a DAA regimen. This individualized approach merit further robust assessment.

Definition of the optimal treatment duration is paramount to reduce costs and improve treatment tolerability without compromising the therapeutic efficacy. Due to the consistently lower response rates with 24 wk of therapy, a 48-wk treatment duration has been recommended as the SOC for genotype 4, similar to genotype 1<sup>[43,48,68]</sup>. A further refinement has been use of early viral responses to allow for shorter treatment durations in highly responsive patients (*i.e.*, response-guided approach). In a double-blind randomized study, Kamal *et al.*<sup>[43]</sup> showed that in patients achieving a complete early viral response (EVR; defined as a negative HCV-RNA at week 12 of treatment), the SVR rate was 86% with a 36-wk therapy and 92% with 48 wk of therapy ( $P = 0.8$ ), whereas PegIFN dose reductions were significantly more common in the 48-wk group. Two randomized controlled trials, one including exclusively genotype 4<sup>[69]</sup> and one including a mixture of both genotype 1 or 4 patients<sup>[70]</sup> have assessed the utility of a



**Table 3** Studies reporting rates of sustained virological response to 48-wk interferon-based combination therapy in patients with hepatitis C genotype 5

Author/Country/Year	No. patients	Regimen	SVR
Legrand-Abravanel/France/2004 <sup>[73]</sup>	12	Standard/Pegylated IFN plus RBV	63.60%
Delwaide/Belgium/2006 <sup>[74]</sup>	6	Standard/Pegylated IFN plus RBV	83%
Bonny/France/2006 <sup>[78]</sup>	87	Standard/Pegylated IFN plus RBV	60%
<sup>1</sup> Antaki/Syria/2008 <sup>[77]</sup>	26	Standard/Pegylated IFN plus RBV	54%
D'Heygere/Belgium/2011 <sup>[79]</sup>	38	Standard/Pegylated IFN plus RBV	55.30%
Karatapanis/Greece/2012 <sup>[29]</sup>	10	Pegylated IFN plus RBV	60%
Antaki/Syria/2012 <sup>[76]</sup>	49	Standard/Pegylated IFN plus RBV	49%
Mauss/Germany/2012	24	Pegylated IFN plus RBV	58%
Papastergiou/Greece/2014 <sup>[81]</sup>	27	Pegylated IFN plus RBV	63%

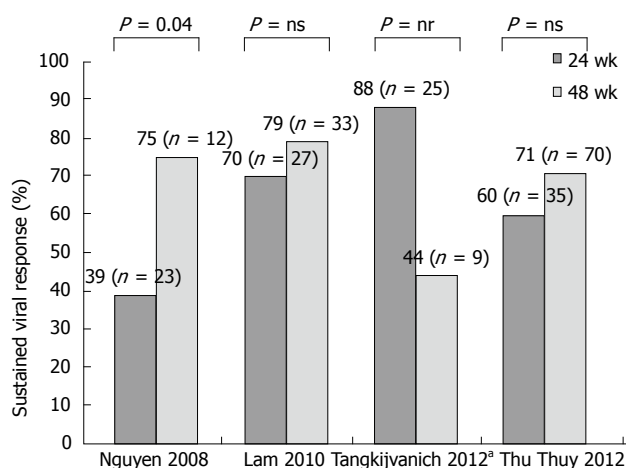
<sup>1</sup>Thirteen out of 26 patients received 24 wk of treatment due to personal, financial or medical reasons. IFN: Interferon; RBV: Ribavirin; SVR: Sustained virological response.

response-guided tailoring of treatment. Based on the results of these studies, a 24- and 36-wk treatment duration have been established as sufficient in patients with a rapid viral response (RVR, defined as negative viral load at week 4 of treatment) and EVR respectively. Contrarily, Ferenci *et al.*<sup>[71]</sup> showed that a 72-wk extended-duration therapy may benefit slow responders; *i.e.*, those non achieving RVR but attaining at least a partial (*i.e.*, a  $\geq 2\log_{10}$  drop in serum HCV-RNA) EVR and a negative HCV-RNA at week 24. Critically, a suboptimal PegIFN alpha-2a dose (135  $\mu\text{g}/\text{wk}$  after week 48) may have compromised SVR rates in this study. Patients with detectable viral load at the end of week 24 are unlikely to respond to treatment, and therefore assessment based on serum HCV-RNA at this time-point may serve as a futility rule, as indicated by its 92.8% negative predictive value on SVR<sup>[53]</sup>. To date, no robust conclusions can be drawn regarding efficacy of PegIFN alpha-2a vs alpha-2b in patients infected with HCV 4<sup>[72]</sup>.

**Hepatitis C virus genotype 5:** Mainly due to its low worldwide prevalence, HCV 5 probably represents the less studied HCV genotype with respect to therapeutic endpoints. Epidemiological reports from France, Belgium, Canada, Syria and Greece argue that patients infected with HCV 5 have specific epidemiological characteristics: they are predominantly females of advanced age and they are characterized by high baseline viremia and advanced hepatic fibrosis<sup>[25,29,73-75]</sup>. Despite presence of these classical negative predictors of treatment response, SVR rates have been reported to 55%-60%<sup>[29,74-81]</sup>, although most of the therapeutic studies on HCV 5 (Table 3) have had inherent limitations, including the retrospective design, small sample and extreme heterogeneity with respect to treatment modalities<sup>[74-76,78]</sup> and patient sampling<sup>[79]</sup>. Currently, a fixed 48-wk course of combined PegIFN and RBV is recommended for patients with HCV 5. However, the intrinsic sensitivity of HCV to combined antiviral therapy, and thus the ideal treatment duration, remains controversial. In a retrospective study by Antaki *et al.*<sup>[77]</sup>, 13/26 patients were treated for 24 wk

due to personal, financial or medical reasons, and no impact of treatment duration (24 wk vs 48 wk) was found on SVR. In support of a 24-wk treatment, some retrospective data<sup>[75,78]</sup> suggested that response of HCV is similar to that observed for genotypes 2 and 3, although this was disputed in more recent studies<sup>[76,79]</sup>. Clearly, extrapolations using other HCV genotypes as reference are not an appropriate basis for treatment standardization. In a prospective, open label, single-arm trial we have evaluated 27 patients with HCV 5 and the SVR was 63%, whereas non-response was mainly due to relapse (26.1%)<sup>[81]</sup>. To our knowledge this is the only prospective therapeutic trial using exclusively a combination of PegIFN and RBV and including only treatment-naïve patients. The most striking finding of our study was the excellent predictive value of early viral responses on SVR: the positive predictive value (PPV) of RVR was 93.8%, whereas the negative predictive value when not achieving EVR was 100%. Based on these data, a response-guided schedule may be a viable option for patients with HCV 5<sup>[82]</sup>. Thus, it merits appropriate consideration in future trials, possibly conducted on a multi-center basis.

**Hepatitis C virus genotype 6:** Small studies, using a combination of either standard interferon<sup>[83,84]</sup> or PegIFN<sup>[85,86]</sup> and RBV have examined treatment outcomes in HCV 6. Overall, SVR rates have ranged to 60%-90%, indicating a more favorable response in comparison to HCV 1 and comparable to that of HCV 2 and 3. Crucially, a favorable IL28B status among Asians patients may have contributed significantly to these good results<sup>[87]</sup>. Apart from baseline viral load and the degree of hepatic fibrosis, which represent classical predictors of treatment response in CHC, other host factors such as age, BMI and adherence to treatment schedule have been identified as relevant in cohorts of patients with HCV 6<sup>[88]</sup>. To date, optimal treatment duration for HCV 6 has been a matter of controversy, with studies comparing a 48- vs a 24-wk regimen providing equivocal results (Figure 2)<sup>[86,89-91]</sup>. Most studies investigating therapeutic outcomes in HCV 6 have applied 48-wk treatment duration<sup>[83-85]</sup>.



**Figure 2** Studies comparing outcomes of 24 vs 48 wk of therapy with pegylated interferon and ribavirin in patients with hepatitis C virus genotype 6. <sup>a</sup>Treatment duration was tailored according to viral response at week 4: Patients with negative hepatitis C virus-RNA received 24 wk of treatment; the remainder received 48 wk. ns: Non-significant; nr: Not reported.

Moreover, in a retrospective analysis by Nguyen *et al.*<sup>[86]</sup>, the SVR rates were significantly lower with a 24-wk treatment schedule (39% vs 75%,  $P = 0.044$ ). However, two randomized controlled trials including 60 and 105 patients have shown non-inferiority with a 24-wk (vs a 48-wk) regimen of PegIFN alpha/2a and RBV<sup>[89,91]</sup>. As in case of other HCV genotypes, individualization of treatment duration based on early viral dynamics may allow for reduction to drug exposure and in treatment costs. In an open-label randomized study by Thu Thuy *et al.*<sup>[91]</sup>, RVR occurred in about 80% of patients and had a 75%-86% PPV on SVR irrespective of treatment duration; nonetheless, among those with no RVR, even a 48-wk treatment achieved low SVR rates (8%). Efficacy of a response-guided approach based on RVR has been evaluated by Tangkijvanich *et al.*<sup>[90]</sup>: in a pilot study including 34 patients with CHC genotype 6, the SVR rate in patients with RVR who underwent a 24-wk treatment was 88%. Based on these data, patients with a RVR may benefit with 24 wk of therapy. On the other hand, interruption of treatment may be the most reasonable option in patients with a detectable viral load by week 12 of therapy (non-EVR), as these patients are unlikely to respond to a 48-wk course<sup>[89,91,92]</sup> whereas there is no data to support they might benefit from longer treatment duration. Additionally to the use of early viral responses, evaluation of baseline parameters such as age, the degree of liver fibrosis, viral load and BMI may further rationalized choice of the optimum treatment duration<sup>[88]</sup>. Larger randomized trials are awaited to optimize treatment schedules for HCV 6.

### Direct-acting antivirals

**First generation protease inhibitors:** Telaprevir and boceprevir are first generation NS3/4 PIs firstly approved in 2011 for patients infected with HCV 1.

Although both drugs are not approved for HCV 4, telaprevir has shown a modest activity against this genotype. A phase IIa trial (study C210) has assessed the activity of telaprevir on early viral dynamics<sup>[21]</sup>. Twenty four patients with HCV 4 were randomized to three groups: telaprevir alone; PegIFN plus RBV; and a triple regimen comprising telaprevir plus PegIFN/RBV. By day 15 of therapy telaprevir monotherapy induced only a 0.77 log<sub>10</sub> decline in HCV-RNA levels (vs 4.77 log<sub>10</sub> for HCV 1). The viral decline was more pronounced (4.32 log<sub>10</sub>) when telaprevir was administered together with PegIFN/RBV indicating a synergic effect. A descriptive subanalysis of the C210 study showed that the most frequent mutation accounting for the limited antiviral efficacy of telaprevir monotherapy was the T54A/T previously described for HCV 1<sup>[93]</sup>. Interestingly, this mutation has limited or no impact on the efficacy of subsequent treatment with dual PegIFN/RBV.

Indeed, emergence of resistant variants has generally precluded monotherapy with first generation PIs. However, use of these agents in conjunction with PegIFN/RBV still depends on interferon sensitivity, requires a high pill burden and a complex treatment algorithm. Moreover, both drugs may enhance or induce a set of considerable side effects. Anemia, neutropenia and dysgeusia are the most common side effects with boceprevir, whereas anemia, skin rash and anorectal symptoms are more frequently associated with telaprevir. Anemia, occurring in about 40%-50% of cases, may lead to discontinuation of treatment despite management with ribavirin dose adaptations, use of erythropoietin alpha or blood transfusions. Skin rash specifically related to telaprevir is generally mild and manageable using emollients and topical corticosteroids, although, in about 5% of cases, a severe life-threatening cutaneous reaction may lead to treatment discontinuation<sup>[94]</sup>.

Better-tolerated new generation DAAs with improved pharmacokinetics (allowing once-daily administration) and favorable resistance profiles (allowing interferon-free, all-oral regimens) were recently approved or await approval. These agents, with activities against HCV 4 to 6, will be discussed below.

**Simeprevir (TMC435):** It is a second generation NS3/4A PI, active against genotypes 1, 2, 4, 5 and 6. It is administered as a once-daily tablet orally and has demonstrated a favorable safety profile and limited drug-drug interactions<sup>[95]</sup>. It was approved in November 2013 by the United States Food and Drug Administration (FDA) and in Japan in September 2013.

RESTORE, a phase III, multicenter, single-arm, open-label study, conducted in France and Belgium, evaluated simeprevir (150 mg once-daily for 12 wk in combination with PegIFN/RBV, followed by 12-36 wk of PegIFN/RBV only) in 107 patients with HCV 4, either naïve or treatment-experienced<sup>[96]</sup>. Overall, SVR

at week 12 was observed in 65.4% of patients, with rates being particularly high among treatment-naïve (82.9%) and prior-relapsers (86.4%). Both these patient groups were eligible to a shorter (totally 24 wk) treatment duration if they achieved a HCV-RNA < 25 IU/mL at week 4 and undetectable at week 12. These response-guide criteria were met by 88.6% of treatment-naïve and 90.9% of prior relapsers; among them 93.5% and 95% respectively achieved a SVR at week 12. Notably, none patient had a detectable Q80K substitution in the NS3 protease sequence at baseline, associated with decreased efficacy in patients with HCV 1a.

Overall, simeprevir has demonstrated favorable safety profile. By pooling data from phase III clinical trials (QUEST-1, QUEST -2 and PROMISE), discontinuation of treatment due to severe adverse events occurred in 2% of patients receiving a simeprevir plus PegIFN/RBV combination<sup>[97-99]</sup>. Rates of adverse events, most commonly fatigue, influenza-like illness, headache, nausea and pruritus, were generally similar between simeprevir/PegIFN/RBV and placebo/PegIFN/RBV groups. Incidence of photosensitivity and rash has been slightly higher with simeprevir (vs placebo), although the vast majority of cases were graded 1/2 in severity. Transient moderate bilirubin increases were noted; however, no clinically relevant cases of hepatotoxicity were recorded.

Recent European guidelines have included a 24-48 wk simeprevir plus PegIFN/RBV combination as an option for HCV 4-related compensated liver disease (including cirrhotics), suggesting interruption of treatment if HCV-RNA levels are  $\geq$  25 IU/mL at week 4, 12 or 24<sup>[100]</sup>.

**Sofosbuvir:** This is a nucleotide inhibitor of NS5B, with a pan-genotypic effect activity and a high barrier to resistance. It is administered as an oral 400 mg tablet/day with no food effect, whereas it has been proven safe and well-tolerated in phase II and III clinical trial including > 2000 patients. It was approved by FDA for HCV 1 in combination with PegIFN/RBV, and in HCV 2 and 3 in interferon-free regimens in December 2013 and in Europe in January 2014.

NEUTRINO, an open-label, single-arm, phase III trial, evaluated a 12-wk regimen, comprising sofosbuvir plus PegIFN/RBV, in 327 treatment-naïve patients with genotypes 1, 4, 5 and 6<sup>[101]</sup>. However, the vast majority (89%) of the patient population had HCV 1. Overall, 27 out of the 28 (96%) patients with HCV 4, all 6 patients with HCV 6 and the single patient with HCV 5 achieved an SVR, 12 wk after the end of treatment. Currently, a sofosbuvir-based triple combination for 12 wk appears as the most efficacious and easy-to-use interferon containing option for the treatment of HCV genotypes 4 to 6, without the risk for selecting resistant variants in case of treatment failure<sup>[100]</sup>. Critically, rates of SVR were relatively lower in cirrhotics in the NEUTRINO trial (80% vs 92% in

patients without cirrhosis), whereas no data with this regimen has been presented in treatment-experienced patients. Thus, it remains unknown whether longer treatment duration may be required for these more difficult-to-treat patient populations.

More recently, promising data have emerged on the efficacy of an interferon-free combination of sofosbuvir plus ribavirin in patients with HCV 4. Ruane *et al.*<sup>[102]</sup> randomized (1:1) 60 patients of Egyptian ancestry (treatment-naïve: 28, treatment-experienced: 32; 23% cirrhotics; 17% with the IL28B CC genotype), stratified by prior treatment status and cirrhosis, to receive 12 or 24 wk of sofosbuvir (400 mg/d) plus RBV (1200 mg/d). After 12 wk of treatment, SVR rates were 11/14 (79%) in treatment-naïve and 10/17 (59%) in treatment-experience patients. However, extending the duration of treatment to 24 wk resulted in higher SVR rates in both treatment-naïve (14/14; 100%) and -experienced (13/15; 87%) groups. Thus, a dual sofosbuvir/ribavirin combination given for 24 wk is currently recommended for HCV 4 patients who are interferon-intolerant or -ineligible<sup>[100]</sup>.

As evident in phase III clinical trials, sofosbuvir in combination with RBV represents a well-tolerated option with rates of treatment discontinuation as low as 1%-2%<sup>[103]</sup>. Drug-related adverse events attributable to RBV such as fatigue, insomnia and anemia were the most common, and headache was also frequent. Unsurprisingly, incidence of adverse effects commonly associated with interferon, such as influenza-like illness and depression, was significantly lower and hematological abnormalities were less prominent among patients who receive sofosbuvir/RBV than among those who receive the standard PegIFN/RBV combination<sup>[101]</sup>. Consistently, health-related quality of life and health utilities of patients have been shown to be only minimally affected by sofosbuvir/RBV irrespectively to treatment duration<sup>[104,105]</sup>.

**Daclatasvir:** This is an HCV NS5A oral PI with a pan-genotypic activity, but a lower barrier to resistance in genotype 1a<sup>[106]</sup>. A recent phase II b double-blind, placebo-controlled study evaluated a triple combination comprising daclatasvir plus PegIFN/RBV including treatment-naïve patients with HCV 1 ( $n = 365$ ) or 4 ( $n = 30$ )<sup>[107]</sup>. Patients were randomly assigned (2:2:1) to daclatasvir 20 mg or 60 mg, or placebo once daily plus PegIFN/RBV. Overall, SVR rates (week 24 post-treatment) were 8/12 (66.7%) in HCV 4 patients receiving 20 mg, 12/12 (100%) in those receiving 60 mg and 3/6 (50%) in patients receiving placebo. Patients on daclatasvir did not have adverse events beyond those typical of PegIFN/RBV.

Based on this preliminary data, a daclatasvir (dose: 60 mg/d) plus PegIFN/RBV regimen has been included as an option for the treatment of genotype 1b and 4 patients<sup>[100]</sup>. The triple combination should be administered for 12 wk. In those who do not achieve an HCV-RNA level < 25 IU/mL at week 4

and undetectable at week 10, all three drugs should be continued for an additional 12 weeks. Conversely, PegIFN/RBV should be continued alone between week 12 and 24 in those who achieve such response<sup>[100]</sup>.

**Other DAAs evaluated in HCV 4:** Phase II trials have evaluated other triple or quadruple drug combinations in patients infected with HCV 4. In the DAUPHINE trial, different dosing schedules of danoprevir boosted with ritonavir plus PegIFN/RBV for 12–24 wk achieved up to 100% of SVR in treatment-naïve patients with HCV 4<sup>[108]</sup>. In yet another phase II b study, 25 HCV-4 patients were assigned to asunaprevir 200 mg or placebo twice daily plus PegIFN/RBV; the SVR rates were 89% in those receiving asunaprevir vs 43% in the placebo group<sup>[109]</sup>. Lastly, two randomized placebo-controlled trials have evaluated use of mericitabine in combination with PegIFN/RBV including patients infected with HCV 4<sup>[110,111]</sup>. In the JUMP-C trial, a 24-wk response-guided combination of mericitabine 1000 mg twice daily plus PegIFN/RBV was well-tolerated and more effective than a standard 48-wk PegIFN/RBV combination<sup>[110]</sup>.

## EMERGING CHALLENGES

While standardization of dual PegIFN/RBV regimens for HCV 4 to 6 is still pending, interferon-based treatment of HCV has been superseded by the introduction of oral DAAs with pan-genotypic activities. These agents are characterized by improved antiviral efficacy and offer the perspective for short-course, all-oral and interferon-free therapies. However, as it is reasonable, most trials evaluating DAAs have focused on the more prevalent and difficult to treat HCV genotype 1. Given obvious difficulties in patient sampling, multicentric efforts may be necessary to assess therapeutic sensitivity, optimize DAA schedules and establish cost-effective response-guided approaches for HCV 4 to 6. Crucially, the very high cost of HCV DAAs is a central barrier to their widespread use, thus interferon-based treatments are likely to continue to have a role as cost-containing options in low- or lower-middle income countries. This is particularly relevant in the case of genotypes 4 to 6 which are mainly based in resource-limited countries; but with large HCV epidemics, hence these genotypes represent > 20% of the global HCV burden. Therefore, unless low-cost DAAs become available, a large population of untreated patients will continue to spread HCV in these countries and worldwide. Furthermore, treatment with DAAs of special patient population (e.g., patients with kidney disease, HIV coinfection, patients undergoing solid organ transplantation) remains a challenge, as few or no data are available.

In conclusion, it seems we need to wait for a while until arrangement of both practical and logistic issues will allow for low-cost, all-oral and interferon-free regimens, at a level to dramatically change the global epidemics of HCV 4 to 6. Until then, efforts to further

rationalize the use of the traditional PegIFN/RBV treatment should continue.

## REFERENCES

- 1 **Lavanchy D.** The global burden of hepatitis C. *Liver Int* 2009; **29** Suppl 1: 74–81 [PMID: 19207969 DOI: 10.1111/j.1478-3231.2008.01934.x]
- 2 **Shepard CW, Finelli L, Alter MJ.** Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; **5**: 558–567 [PMID: 16122679 DOI: 10.1016/S1473-3099(05)70216-4]
- 3 **McOmish F, Yap PL, Dow BC, Follett EA, Seed C, Keller AJ, Cobain TJ, Krusius T, Kolho E, Naukkarinen R.** Geographical distribution of hepatitis C virus genotypes in blood donors: an international collaborative survey. *J Clin Microbiol* 1994; **32**: 884–892 [PMID: 7913097]
- 4 **Robertson B, Myers G, Howard C, Brettin T, Bukh J, Gaschen B, Gojobori T, Maertens G, Mizokami M, Nainan O, Netesov S, Nishioka K, Shin I T, Simmonds P, Smith D, Stuyver L, Weiner A.** Classification, nomenclature, and database development for hepatitis C virus (HCV) and related viruses: proposals for standardization. International Committee on Virus Taxonomy. *Arch Virol* 1998; **143**: 2493–2503 [PMID: 9930205]
- 5 **Abdulkarim AS, Zein NN, Germer JJ, Kolbert CP, Kabbani L, Krajnik KL, Hola A, Agha MN, Tourogman M, Persing DH.** Hepatitis C virus genotypes and hepatitis G virus in hemodialysis patients from Syria: identification of two novel hepatitis C virus subtypes. *Am J Trop Med Hyg* 1998; **59**: 571–576 [PMID: 9790432]
- 6 **Al-Kubaisy WA, Niazi AD, Kubba K.** History of miscarriage as a risk factor for hepatitis C virus infection in pregnant Iraqi women. *East Mediterr Health J* 2002; **8**: 239–244 [PMID: 15339110]
- 7 **Njoum R, Pasquier C, Ayoub A, Gessain A, Froment A, Mfoupouendoun J, Pouillot R, Dubois M, Sandres-Sauné K, Thonnnon J, Izopet J, Nerrienet E.** High rate of hepatitis C virus infection and predominance of genotype 4 among elderly inhabitants of a remote village of the rain forest of South Cameroon. *J Med Virol* 2003; **71**: 219–225 [PMID: 12938196 DOI: 10.1002/jmv.10473]
- 8 **Oni AO, Harrison TJ.** Genotypes of hepatitis C virus in Nigeria. *J Med Virol* 1996; **49**: 178–186 [PMID: 8818962 DOI: 10.1002/(SICI)1096-9071(199607)49:3<178::AID-JMV4>3.0.CO;2-1]
- 9 **Ramias S, Koussa S, Taher A, Haraki S, Klayme S, Sarkis D, Naman R.** Hepatitis-C-virus genotypes and hepatitis-G-virus infection in Lebanese thalassaemics. *Ann Trop Med Parasitol* 2002; **96**: 197–202 [PMID: 12080981 DOI: 10.1179/000349802125000439]
- 10 **Ray SC, Arthur RR, Carella A, Bukh J, Thomas DL.** Genetic epidemiology of hepatitis C virus throughout Egypt. *J Infect Dis* 2000; **182**: 698–707 [PMID: 10950762]
- 11 **Shobokshi OA, Serebour FE, Skakni L, Al-Saffy YH, Ahdal MN.** Hepatitis C genotypes and subtypes in Saudi Arabia. *J Med Virol* 1999; **58**: 44–48 [PMID: 10223544 DOI: 10.1002/(SICI)1096-9071(199905)58:1<44::AID-JMV6>3.0.CO;2-U]
- 12 **Xu LZ, Larzul D, Delaporte E, Bréchet C, Kremsdorf D.** Hepatitis C virus genotype 4 is highly prevalent in central Africa (Gabon). *J Gen Virol* 1994; **75** (Pt 9): 2393–2398 [PMID: 8077938]
- 13 **Alter MJ, Kruszon-Moran D, Nainan OV, McQuillan GM, Gao F, Moyer LA, Kaslow RA, Margolis HS.** The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N Engl J Med* 1999; **341**: 556–562 [PMID: 10451460 DOI: 10.1056/NEJM199908193410802]
- 14 **Zein NN, Rakela J, Krawitt EL, Reddy KR, Tominaga T, Persing DH.** Hepatitis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. Collaborative Study Group. *Ann Intern Med* 1996; **125**: 634–639 [PMID: 8849147]
- 15 **Payan C, Roudot-Thoraval F, Marcellin P, Bled N, Duverlie G, Fouchard-Hubert I, Trimoulet P, Couzigou P, Cointe D, Chaput C, Henquell C, Abergel A, Pawlotsky JM, Hezode C, Coudé M, Blanchi A, Alain S, Loustaud-Ratti V, Chevallier P, Trepo C, Gerolami V, Portal I, Halfon P, Bourlière M, Bogard M, Plouvier**



- E, Laffont C, Agius G, Silvain C, Brodard V, Thieffn G, Buffet-Janvresse C, Riachi G, Grattard F, Bourlet T, Stoll-Keller F, Doffoel M, Izopet J, Barange K, Martinot-Peignoux M, Branger M, Rosenberg A, Sogni P, Chaix ML, Pol S, Thibault V, Opolon P, Charrois A, Serfaty L, Fouqueray B, Grange JD, Lefrère JJ, Lunel-Fabiani F. Changing of hepatitis C virus genotype patterns in France at the beginning of the third millenium: The GEMHEP GenoCII Study. *J Viral Hepat* 2005; **12**: 405-413 [PMID: 15985012 DOI: 10.1111/j.1365-2893.2005.00605.x]
- 16 **Argentini C**, Dettori S, Villano U, Guadagnino V, Infantolino D, Dentico P, Coppola RC, Rapicetta M. Molecular characterisation of HCV genotype 4 isolates circulating in Italy. *J Med Virol* 2000; **62**: 84-90 [PMID: 10935993 DOI: 10.1002/1096-9071(200009)62:1<84::AID-JMV13>3.0.CO;2-E]
  - 17 **Esteban JI**, Saulea S, Quer J. The changing epidemiology of hepatitis C virus infection in Europe. *J Hepatol* 2008; **48**: 148-162 [PMID: 18022726 DOI: 10.1016/j.jhep.2007.07.033]
  - 18 **Katsoulidou A**, Sypsa V, Tassopoulos NC, Boletis J, Karafoulidou A, Ketikoglou I, Tsantoulas D, Vafiadi I, Hatzis G, Skoutelis A, Akriavidis E, Vasiliadis T, Kitis G, Magiorkinis G, Hatzakis A. Molecular epidemiology of hepatitis C virus (HCV) in Greece: temporal trends in HCV genotype-specific incidence and molecular characterization of genotype 4 isolates. *J Viral Hepat* 2006; **13**: 19-27 [PMID: 16364078 DOI: 10.1111/j.1365-2893.2005.00649.x]
  - 19 **Matera G**, Lamberti A, Quirino A, Focà D, Giancotti A, Barreca GS, Guadagnino V, Liberto MC. Changes in the prevalence of hepatitis C virus (HCV) genotype 4 in Calabria, Southern Italy. *Diagn Microbiol Infect Dis* 2002; **42**: 169-173 [PMID: 11929687 DOI: 10.1054/dm.2002.3509]
  - 20 **Sánchez-Quijano A**, Abad MA, Torronteras R, Rey C, Pineda JA, Leal M, Macías J, Lissen E. Unexpected high prevalence of hepatitis C virus genotype 4 in Southern Spain. *J Hepatol* 1997; **27**: 25-29 [PMID: 9252069 DOI: 10.1016/S0168-8278(97)80275-9]
  - 21 **Benhamou Y**, Moussalli J, Ratzu V, Lebray P, De Backer K, De Meyer S, Ghys A, Luo D, Picchio GR, Beumont M. Telaprevir activity in treatment-naïve patients infected hepatitis C virus genotype 4: a randomized trial. *J Infect Dis* 2013; **208**: 1000-1007 [PMID: 23801602 DOI: 10.1093/infdis/jit274jit274]
  - 22 **Ohno T**, Mizokami M, Tibbs CJ, Ohba K, Suzuki K, Wu RR, Nouri-Aria KT, Williams R. New genotype of hepatitis C virus in South Africa. *J Med Virol* 1994; **42**: 409-413 [PMID: 8046432]
  - 23 **Smuts HE**, Kannemeyer J. Genotyping of hepatitis C virus in South Africa. *J Clin Microbiol* 1995; **33**: 1679-1681 [PMID: 7650216]
  - 24 **Ansaldi F**, Bruzzone B, Salmaso S, Rota MC, Durando P, Gasparini R, Icardi G. Different seroprevalence and molecular epidemiology patterns of hepatitis C virus infection in Italy. *J Med Virol* 2005; **76**: 327-332 [PMID: 15902713 DOI: 10.1002/jmv.20376]
  - 25 **Antaki N**, Haddad N, Kebbewar K, Abdelwahab J, Hamed O, Aaraj R, Alhaj N, Haffar S, Assil M, Ftayeh M, Assaad F, Doghman D, Ali T, Nassereldine M, Ali A, Antaki F. The unexpected discovery of a focus of hepatitis C virus genotype 5 in a Syrian province. *Epidemiol Infect* 2009; **137**: 79-84 [PMID: 18346288 DOI: 10.1017/S095026880800054XS095026880800054X]
  - 26 **Henquell C**, Cartau C, Abergel A, Laurichesse H, Regagnon C, De Champs C, Bailly JL, Peigue-Lafeuille H. High prevalence of hepatitis C virus type 5 in central France evidenced by a prospective study from 1996 to 2002. *J Clin Microbiol* 2004; **42**: 3030-3035 [PMID: 15243055 DOI: 10.1128/JCM.42.7.3030-3035.200442/7/3030]
  - 27 **Jover R**, Pérez-Serra J, de Vera F, Alamo JM, Muñoz C, Yago C, Martínez-Ramírez R, Vidal JV. Infection by genotype 5a of HCV in a district of southeast Spain. *Am J Gastroenterol* 2001; **96**: 3042-3043 [PMID: 11693355 DOI: 10.1111/j.1572-0241.2001.04695.x]
  - 28 **Verbeek J**, Maes P, Lemey P, Pybus OG, Wollants E, Song E, Nevens F, Fevery J, Delpont W, Van der Merwe S, Van Ranst M. Investigating the origin and spread of hepatitis C virus genotype 5a. *J Virol* 2006; **80**: 4220-4226 [PMID: 16611881 DOI: 10.1128/JVI.80.9.4220-4226.2006]
  - 29 **Karatapanis S**, Tsoplou P, Papastergiou V, Vasiageorgi A, Stampori M, Saitis I, Tsitsopoulos E, Ligos P, Skorda L, Ketikoglou I, Goulis I. Hepatitis C virus genotyping in Greece: unexpected high prevalence of genotype 5a in a Greek island. *J Med Virol* 2012; **84**: 223-228 [PMID: 22170541 DOI: 10.1002/jmv.22249]
  - 30 **Nguyen MH**, Keeffe EB. Chronic hepatitis C: genotypes 4 to 9. *Clin Liver Dis* 2005; **9**: 411-426, vi [PMID: 16023974 DOI: 10.1016/j.cld.2005.05.010]
  - 31 **Oh HB**, Kim SO, Cha CH, Hong SP, Folk WR, Kim KM, Suh DJ. Identification of hepatitis C virus genotype 6 in Korean patients by analysis of 5' untranslated region using a matrix assisted laser desorption/ionization time of flight-based assay, restriction fragment mass polymorphism. *J Med Virol* 2008; **80**: 1712-1719 [PMID: 18712825 DOI: 10.1002/jmv.21162]
  - 32 **Peng JS**, Wang X, Liu MQ, Zhou DJ, Gong J, Xu HM, Chen JP, Zhu HH, Zhou W, Ho WZ. Genetic variation of hepatitis C virus in a cohort of injection heroin users in Wuhan, China. *Virus Res* 2008; **135**: 191-196 [PMID: 18353479 DOI: 10.1016/j.virusres.2008.01.017]
  - 33 **Prescott LE**, Simmonds P, Lai CL, Chan NK, Pike I, Yap PL, Lin CK. Detection and clinical features of hepatitis C virus type 6 infections in blood donors from Hong Kong. *J Med Virol* 1996; **50**: 168-175 [PMID: 8915883 DOI: 10.1002/(SICI)1096-9071(199610)50:2<168::AID-JMV10>3.0.CO;2-I]
  - 34 **Wong DA**, Tong LK, Lim W. High prevalence of hepatitis C virus genotype 6 among certain risk groups in Hong Kong. *Eur J Epidemiol* 1998; **14**: 421-426 [PMID: 9744672]
  - 35 **Zhang YY**, Lok AS, Chan WZ, Widell A. Greater diversity of hepatitis C virus genotypes found in Hong Kong than in mainland China. *J Clin Microbiol* 1995; **33**: 2931-2934 [PMID: 8576348]
  - 36 **Kronenberg B**, Zeuzem S. New developments in HCV therapy. *J Viral Hepat* 2012; **19** Suppl 1: 48-51 [PMID: 22233414 DOI: 10.1111/j.1365-2893.2011.01526.x]
  - 37 **Cha A**, Budovich A. Sofosbuvir: a new oral once-daily agent for the treatment of hepatitis C virus infection. *P T* 2014; **39**: 345-352 [PMID: 24883006]
  - 38 **Kamal SM**, Madwar MA, Peters T, Fawzy R, Rasenack J. Interferon therapy in patients with chronic hepatitis C and schistosomiasis. *J Hepatol* 2000; **32**: 172-174 [PMID: 10673085]
  - 39 **Zylberberg H**, Chaix ML, Bréchet C. Infection with hepatitis C virus genotype 4 is associated with a poor response to interferon-alpha. *Ann Intern Med* 2000; **132**: 845-846 [PMID: 10819720]
  - 40 **Derbala MF**, Al Kaabi SR, El Dweik NZ, Pasic F, Butt MT, Yakoob R, Al-Marri A, Amer AM, Morad N, Bener A. Treatment of hepatitis C virus genotype 4 with peginterferon alfa-2a: impact of bilharziasis and fibrosis stage. *World J Gastroenterol* 2006; **12**: 5692-5698 [PMID: 17007024]
  - 41 **Esmat G**, Mohamed MK, Abdel Hamid M, Zalata K, Khattab M, El Batanony M, Abouzied AM, El Raziky M, Shaheen AM, Ismail A, Strickland GT, Fix A. The impact of steatosis on baseline characteristic and end of treatment response for chronic hepatitis (C) genotype 4 patients treated with interferon. *J Hepatol* 2003; **38** (Suppl. 2): 139
  - 42 **Hasan F**, Asker H, Al-Khaldi J, Siddique I, Al-Ajmi M, Owaid S, Varghese R, Al-Nakib B. Peginterferon alfa-2b plus ribavirin for the treatment of chronic hepatitis C genotype 4. *Am J Gastroenterol* 2004; **99**: 1733-1737 [PMID: 15330911 DOI: 10.1111/j.1572-0241.2004.40077.x]
  - 43 **Kamal SM**, El Tawil AA, Nakano T, He Q, Rasenack J, Hakam SA, Saleh WA, Ismail A, Aziz AA, Madwar MA. Peginterferon [alpha]-2b and ribavirin therapy in chronic hepatitis C genotype 4: impact of treatment duration and viral kinetics on sustained virological response. *Gut* 2005; **54**: 858-866 [PMID: 15888797 DOI: 10.1136/gut.2004.057182]
  - 44 **Shobokshi O**, Serebour F, Skakni L, Tantawi T, Dinish M, At Quwaiz M. Efficacy of pegylated (40 kDa) IFN alfa-2a (PEGASYS) plus ribavirin in the treatment of hepatitis C genotype 4 chronic active patients in Saudi Arabia. *J Hepatol* 2002; **36** (Suppl. 1): 129
  - 45 **Thakeb F**, Omar M, Bilharz T, Awady M, Isshak S. Randomized controlled trial of peginterferon alfa- 2a plus ribavirin for chronic hepatitis C virus-genotype 4 among Egyptian patients. *Hepatology*

- 2003; **38**: 278A
- 46 **Urquijo JJ**, Diago M, Boadas J, Planas R, Solá R, Del Olmo JA, Crespo J, Erdozain JC, Antón MD, Arocena C, Suarez D, Giné J, Barrera JM, Gracia-Samaniego J, Perez R, Dalmau B, Montoro M. Safety and efficacy of treatment with pegylated interferon alpha-2a with ribavirin in chronic hepatitis C genotype 4. *Ann Hepatol* 2013; **12**: 30-35 [PMID: 23293191]
  - 47 **Diago M**, Hassanein T, Rodés J, Ackrill AM, Sedarati F. Optimized virologic response in hepatitis C virus genotype 4 with peginterferon-alpha2a and ribavirin. *Ann Intern Med* 2004; **140**: 72-73 [PMID: 14706990]
  - 48 **El-Zayadi AR**, Attia M, Barakat EM, Badran HM, Hamdy H, El-Tawil A, El-Nakeeb A, Selim O, Saied A. Response of hepatitis C genotype-4 naïve patients to 24 weeks of Peg-interferon-alpha2b/ribavirin or induction-dose interferon-alpha2b/ribavirin/amantadine: a non-randomized controlled study. *Am J Gastroenterol* 2005; **100**: 2447-2452 [PMID: 16279899 DOI: 10.1111/j.1572-0241.2005.00253.x]
  - 49 **Khatab M**, Eslam M, Sharwae MA, Shatat M, Ali A, Hamdy L. Insulin resistance predicts rapid virologic response to peginterferon/ribavirin combination therapy in hepatitis C genotype 4 patients. *Am J Gastroenterol* 2010; **105**: 1970-1977 [PMID: 20234345 DOI: 10.1038/ajg.2010.110]
  - 50 **Khuroo MS**, Khuroo MS, Dahab ST. Meta-analysis: a randomized trial of peginterferon plus ribavirin for the initial treatment of chronic hepatitis C genotype 4. *Aliment Pharmacol Ther* 2004; **20**: 931-938 [PMID: 15521839 DOI: 10.1111/j.1365-2036.2004.02208.x]
  - 51 **Trapero-Marugan M**, Moreno-Monteagudo JA, Garcia-Buey L, Borque MJ, Medina J, Garcia-Sanchez A, Moreno-Otero R. Clinical and pathological characteristics and response to combination therapy of genotype 4 chronic hepatitis C patients: experience from a spanish center. *J Chemother* 2007; **19**: 423-427 [PMID: 17855187 DOI: 10.1179/joc.2007.19.4.423]
  - 52 **Roulot D**, Bourcier V, Grando V, Deny P, Baazia Y, Fontaine H, Bailly F, Castera L, De Ledinghen V, Marcellin P, Poupon R, Bourlière M, Zarski JP, Roudot-Thoraval F. Epidemiological characteristics and response to peginterferon plus ribavirin treatment of hepatitis C virus genotype 4 infection. *J Viral Hepat* 2007; **14**: 460-467 [PMID: 17576387 DOI: 10.1111/j.1365-2893.2006.00823.x]
  - 53 **Elefsiniotis IS**, Pavlidis C, Dimitroulopoulos D, Vezali E, Mihas C, Mariolis-Sapsakos T, Koutsounas S, Paraskevas E, Saroglou G. Differential viral kinetics in treated genotype 4 chronic hepatitis C patients according to ethnicity. *J Viral Hepat* 2009; **16**: 738-742 [PMID: 19413697 DOI: 10.1111/j.1365-2893.2009.01134.x]
  - 54 **Moucari R**, Ripault MP, Martinot-Peignoux M, Voitot H, Cardoso AC, Stern C, Boyer N, Maylin S, Nicolas-Chanoine MH, Vidaud M, Valla D, Bedossa P, Marcellin P. Insulin resistance and geographical origin: major predictors of liver fibrosis and response to peginterferon and ribavirin in HCV-4. *Gut* 2009; **58**: 1662-1669 [PMID: 19671541 DOI: 10.1136/gut.2009.185074]
  - 55 **Taira S**, Hizume M, Note I, Sugimoto J, Okita R. A template for forward planning in prostate cancer treatment: conformal irradiation with segmental intensity-modulation. *Igaku Butsuri* 2003; **23**: 59-64 [PMID: 12832866]
  - 56 **Thio CL**, Thomas DL, Goedert JJ, Vlahov D, Nelson KE, Hilgartner MW, O'Brien SJ, Karacki P, Marti D, Astemborski J, Carrington M. Racial differences in HLA class II associations with hepatitis C virus outcomes. *J Infect Dis* 2001; **184**: 16-21 [PMID: 11398104 DOI: 10.1086/321005]
  - 57 **Papastergiou V**, Dimitroulopoulos D, Skorda L, Ligos P, Ketikoglou I, Kostas N, Karatapanis S. Predictors of sustained virological response in Greek and Egyptian patients with hepatitis C genotype 4: does ethnicity matter? *J Med Virol* 2012; **84**: 1217-1223 [PMID: 22711349 DOI: 10.1002/jmv.23324]
  - 58 **Asselah T**, De Muynck S, Broët P, Masliah-Planchon J, Blanluet M, Bièche I, Lapalus M, Martinot-Peignoux M, Lada O, Estrabaud E, Zhang Q, El Ray A, Vidaud D, Ripault MP, Boyer N, Bedossa P, Valla D, Vidaud M, Marcellin P. IL28B polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis C. *J Hepatol* 2012; **56**: 527-532 [PMID: 21951981 DOI: 10.1016/j.jhep.2011.09.008]
  - 59 **Derbala M**, Rizk NM, Al-Kaabi S, John A, Sharma M, El-dweik N, Yakoub R, Pasic F, Almohanadi M, Alejji K, Abdelmola A, Butt M. The predictive value of IL28B rs12979860, rs11881222 and rs8099917 polymorphisms and IP-10 in the therapeutic response of Egyptian genotype 4 patients. *Virology* 2013; **444**: 292-300 [PMID: 23866096 DOI: 10.1016/j.virol.2013.06.025]
  - 60 **Gad RR**, Males S, El Makhzangy H, Shouman S, Hasan A, Attala M, El Hoseiny M, Zalata K, Abdel-Hamid M, Fontanet A, Mohamed MK, Esmat G. Predictors of a sustained virological response in patients with genotype 4 chronic hepatitis C. *Liver Int* 2008; **28**: 1112-1119 [PMID: 18397226 DOI: 10.1111/j.1478-3231.2008.01750.x]
  - 61 **Martín-Carbonero L**, Puoti M, García-Samaniego J, De Luca A, Losada E, Quinzan G, Bruno R, Mariño A, González M, Núñez M, Soriano V. Response to pegylated interferon plus ribavirin in HIV-infected patients with chronic hepatitis C due to genotype 4. *J Viral Hepat* 2008; **15**: 710-715 [PMID: 18637070 DOI: 10.1111/j.1365-2893.2008.01015.x]
  - 62 **Kamal S**, Madwar M, Bianchi L, Tawil AE, Fawzy R, Peters T, Rasenack JW. Clinical, virological and histopathological features: long-term follow-up in patients with chronic hepatitis C co-infected with S. mansoni. *Liver* 2000; **20**: 281-289 [PMID: 10959806]
  - 63 **Antaki N**, Bibert S, Kebbewar K, Asaad F, Baroudi O, Alideeb S, Hadad M, Abboud D, Sabah H, Bochud PY, Negro F. IL28B polymorphisms predict response to therapy among chronic hepatitis C patients with HCV genotype 4. *J Viral Hepat* 2013; **20**: 59-64 [PMID: 23231085 DOI: 10.1111/j.1365-2893.2012.01621.x]
  - 64 **Khatab M**, Emad M, Abdelaleem A, Eslam M, Atef R, Shaker Y, Hamdy L. Pioglitazone improves virological response to peginterferon alpha-2b/ribavirin combination therapy in hepatitis C genotype 4 patients with insulin resistance. *Liver Int* 2010; **30**: 447-454 [PMID: 19919569 DOI: 10.1111/j.1478-3231.2009.02171.x]
  - 65 **Hwang SJ**, Lee SD. Hepatic steatosis and hepatitis C: Still unhappy bedfellows? *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 96-101 [PMID: 21199519 DOI: 10.1111/j.1440-1746.2010.06542.x]
  - 66 **Tsochatzis E**, Papatheodoridis GV, Manesis EK, Chrysanthos N, Kafri G, Petraki K, Hadziyannis E, Pandelidaki H, Zafiropoulou R, Savvas S, Koskinas J, Archimandritis AJ. Hepatic steatosis in genotype 4 chronic hepatitis C is mainly because of metabolic factors. *Am J Gastroenterol* 2007; **102**: 634-641 [PMID: 17222326 DOI: 10.1111/j.1572-0241.2006.01025.x]
  - 67 **Boglione L**, Cusato J, De Nicolò A, Cariti G, Allegra S, Ghisetti V, Di Perri G, D'Avolio A. Identification of naïve HVC-4 patients who may be treated with pegylated-interferon and ribavirin according to IL28B polymorphisms. *Antiviral Res* 2014; **106**: 105-110 [PMID: 24726902 DOI: 10.1016/j.antiviral.2014.03.016]
  - 68 **Fried MW**, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçalves FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982 [PMID: 12324553 DOI: 10.1056/NEJMoa020047]
  - 69 **Kamal SM**, El Kamary SS, Shardell MD, Hashem M, Ahmed IN, Muhammadi M, Sayed K, Moustafa A, Hakem SA, Ibrahim A, Moniem M, Mansour H, Abdelaziz M. Pegylated interferon alpha-2b plus ribavirin in patients with genotype 4 chronic hepatitis C: The role of rapid and early virologic response. *Hepatology* 2007; **46**: 1732-1740 [PMID: 17943989 DOI: 10.1002/hep.21917]
  - 70 **Ferenci P**, Laferl H, Scherzer TM, Gschwantler M, Maieron A, Brunner H, Stauber R, Bischof M, Bauer B, Datz C, Löschenberger K, Formann E, Staufer K, Steindl-Munda P. Peginterferon alfa-2a and ribavirin for 24 weeks in hepatitis C type 1 and 4 patients with rapid virological response. *Gastroenterology* 2008; **135**: 451-458 [PMID: 18503773 DOI: 10.1053/j.gastro.2008.04.015]
  - 71 **Ferenci P**, Laferl H, Scherzer TM, Maieron A, Hofer H, Stauber R, Gschwantler M, Brunner H, Wenisch C, Bischof M, Strasser M, Datz C, Vogel W, Löschenberger K, Steindl-Munda P. Peginterferon

- alfa-2a/ribavirin for 48 or 72 weeks in hepatitis C genotypes 1 and 4 patients with slow virologic response. *Gastroenterology* 2010; **138**: 503-512, 512.e1 [PMID: 19909752 DOI: 10.1053/j.gastro.2009.10.058]
- 72 **Esmat G**, El Kassas M, Hassany M, Gamil M, El Raziky M. Optimizing treatment for HCV genotype 4: PEG-IFN alfa 2a vs. PEG-IFN alfa 2b; the debate continues. *Liver Int* 2014; **34** Suppl 1: 24-28 [PMID: 24373075 DOI: 10.1111/liv.12397]
- 73 **Bernier L**, Willems B, Delage G, Murphy DG. Identification of numerous hepatitis C virus genotypes in Montreal, Canada. *J Clin Microbiol* 1996; **34**: 2815-2818 [PMID: 8897188]
- 74 **Delwaide J**, Gerard C, Reenaers C, Vaira D, Bastens B, Bataille C, Servais B, Maes B, Belaiche J, Hepatotropes GL. Hepatitis C virus genotype 5 in southern Belgium: epidemiological characteristics and response to therapy. *Dig Dis Sci* 2005; **50**: 2348-2351 [PMID: 16416187 DOI: 10.1007/s10620-005-3060-4]
- 75 **Legrand-Abravanel F**, Sandres-Sauné K, Barange K, Alric L, Moreau J, Desmorat P, Vinel JP, Izopet J. Hepatitis C virus genotype 5: epidemiological characteristics and sensitivity to combination therapy with interferon-alpha plus ribavirin. *J Infect Dis* 2004; **189**: 1397-1400 [PMID: 15073676 DOI: 10.1086/382544]
- 76 **Antaki N**, Bibert S, Kebbewar K, Asaad F, Baroudi O, Alideeb S, Hadad M, Abboud D, Sabah H, Bochud PY, Negro F. IL28B polymorphisms do not predict response to therapy in chronic hepatitis C with HCV genotype 5. *Gut* 2012; **61**: 1640-1641 [PMID: 22345656 DOI: 10.1136/gutjnl-2012-302019]
- 77 **Antaki N**, Hermes A, Hadad M, Ftayeh M, Antaki F, Abdo N, Kebbewar K. Efficacy of interferon plus ribavirin in the treatment of hepatitis C virus genotype 5. *J Viral Hepat* 2008; **15**: 383-386 [PMID: 18086180 DOI: 10.1111/j.1365-2893.2007.00946.x]
- 78 **Bonny C**, Fontaine H, Poynard T, Hézode C, Larrey D, Marcellin P, Bourlière M, Bronowicki JP, Merle P, Zarski JP, Sapcey T, Guillemard C, Ughetto S, Henquell C, Nicolas C, Roche C, Randl K, Bommelaer G, Abergel A. Effectiveness of interferon plus ribavirin combination in the treatment of naive patients with hepatitis C virus type 5. A French multicentre retrospective study. *Aliment Pharmacol Ther* 2006; **24**: 593-600 [PMID: 16907892 DOI: 10.1111/j.1365-2036.2006.03018.x]
- 79 **D'Heygere F**, George C, Van Vlierberghe H, Decaestecker J, Nakad A, Adler M, Delwaide J, Laureys A, Nevens F. Efficacy of interferon-based antiviral therapy in patients with chronic hepatitis C infected with genotype 5: a meta-analysis of two large prospective clinical trials. *J Med Virol* 2011; **83**: 815-819 [PMID: 21412790 DOI: 10.1002/jmv.22049]
- 80 **Mauss S**, Berger F, Vogel M, Pfeiffer-Vornkahl H, Alshuth U, Rockstroh JK, Niederau C, Hüppe D. Treatment results of chronic hepatitis C genotype 5 and 6 infections in Germany. *Z Gastroenterol* 2012; **50**: 441-444 [PMID: 22581697 DOI: 10.1055/s-0031-1282072]
- 81 **Papastergiou V**, Skorda L, Ligos P, Stampori M, Ntetskas G, Papakonstantinou L, Prodromidou K, Karatapanis S. Hepatitis C virus genotype 5: prospective evaluation of peginterferon/ribavirin treatment efficacy and predictive value of on-treatment virological responses for sustained virological response. *J Clin Gastroenterol* 2014; **48**: 160-165 [PMID: 24100748 DOI: 10.1097/MCG.0b013e3182a1789c]
- 82 **Papastergiou V**, Karatapanis S. Letter: Response-guided treatment of hepatitis C virus genotype 5 may be feasible. *Aliment Pharmacol Ther* 2014; **39**: 1337-1338 [PMID: 24803251 DOI: 10.1111/apt.12702]
- 83 **Dev AT**, McCaw R, Sundararajan V, Bowden S, Sievert W. Southeast Asian patients with chronic hepatitis C: the impact of novel genotypes and race on treatment outcome. *Hepatology* 2002; **36**: 1259-1265 [PMID: 12395338 DOI: 10.1053/jhep.2002.36781]
- 84 **Hui CK**, Yuen MF, Sablon E, Chan AO, Wong BC, Lai CL. Interferon and ribavirin therapy for chronic hepatitis C virus genotype 6: a comparison with genotype 1. *J Infect Dis* 2003; **187**: 1071-1074 [PMID: 12660921 DOI: 10.1086/368217]
- 85 **Fung J**, Lai CL, Hung I, Young J, Cheng C, Wong D, Yuen MF. Chronic hepatitis C virus genotype 6 infection: response to pegylated interferon and ribavirin. *J Infect Dis* 2008; **198**: 808-812 [PMID: 18657036 DOI: 10.1086/591252]
- 86 **Nguyen MH**, Trinh HN, Garcia R, Nguyen G, Lam KD, Keffe EB. Higher rate of sustained virologic response in chronic hepatitis C genotype 6 treated with 48 weeks versus 24 weeks of peginterferon plus ribavirin. *Am J Gastroenterol* 2008; **103**: 1131-1135 [PMID: 18477343 DOI: 10.1111/j.1572-0241.2008.01793.x]
- 87 **Chuang WL**, Yu ML. Host factors determining the efficacy of hepatitis C treatment. *J Gastroenterol* 2013; **48**: 22-30 [PMID: 23104468 DOI: 10.1007/s00535-012-0669-x]
- 88 **Bunchorntavakul C**, Chavalitthamrong D, Tanwandee T. Hepatitis C genotype 6: A concise review and response-guided therapy proposal. *World J Hepatol* 2013; **5**: 496-504 [PMID: 24073301 DOI: 10.4254/wjh.v5.i9.496]
- 89 **Lam KD**, Trinh HN, Do ST, Nguyen TT, Garcia RT, Nguyen T, Phan QQ, Nguyen HA, Nguyen KK, Nguyen LH, Nguyen MH. Randomized controlled trial of pegylated interferon-alfa 2a and ribavirin in treatment-naïve chronic hepatitis C genotype 6. *Hepatology* 2010; **52**: 1573-1580 [PMID: 21038410 DOI: 10.1002/hep.23889]
- 90 **Tangkijvanich P**, Komolmit P, Mahachai V, Poovorawan K, Akkarathamrongsin S, Poovorawan Y. Response-guided therapy for patients with hepatitis C virus genotype 6 infection: a pilot study. *J Viral Hepat* 2012; **19**: 423-430 [PMID: 22571904 DOI: 10.1111/j.1365-2893.2011.01566.x]
- 91 **Thu Thuy PT**, Bunchorntavakul C, Tan Dat H, Rajender Reddy K. A randomized trial of 48 versus 24 weeks of combination pegylated interferon and ribavirin therapy in genotype 6 chronic hepatitis C. *J Hepatol* 2012; **56**: 1012-1018 [PMID: 22266603 DOI: 10.1016/j.jhep.2011.12.020]
- 92 **Zhou YQ**, Wang XH, Hong GH, Zhu Y, Zhang XQ, Hu YJ, Mao Q. Twenty-four weeks of pegylated interferon plus ribavirin effectively treat patients with HCV genotype 6a. *J Viral Hepat* 2011; **18**: 595-600 [PMID: 21105968 DOI: 10.1111/j.1365-2893.2010.01373.x]
- 93 **De Meyer S**, Ghys A, Dierynck I, Beumont M, Luo D, Picchio G. Virologic characterization of genotype 4 hepatitis C virus variants in patients treated with telaprevir. *Virol J* 2014; **11**: 93 [PMID: 24886541 DOI: 10.1186/1743-422X-11-93]
- 94 **Hézode C**. Boceprevir and telaprevir for the treatment of chronic hepatitis C: safety management in clinical practice. *Liver Int* 2012; **32** Suppl 1: 32-38 [PMID: 22212569 DOI: 10.1111/j.1478-3231.2011.02707.x]
- 95 **Gaetano JN**. Benefit-risk assessment of new and emerging treatments for hepatitis C: focus on simeprevir and sofosbuvir. *Drug Healthc Patient Saf* 2014; **6**: 37-45 [PMID: 24729731 DOI: 10.2147/DHPS.S43304]
- 96 **Moreno C**, Hezode C, Marcellin P, Bourgeois S, Francque S, Samuel D, Zoulim F, Grange JD, Lenz O, Ouwerkerk-Mahadevan S, Peeters M, Beumont-Mauviel M, Jessner W. Once-daily simeprevir (TMC435) with peginterferon/ribavirin in treatment-naïve or treatment-experienced chronic HCV genotype 4-infected patients: final results of a phase III trial. *J Hepatol* 2014; **60** (Suppl 1): S535
- 97 **Forns X**, Lawitz E, Zeuzem S, Gane E, Bronowicki JP, Andreone P, Horban A, Brown A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, Scott J, De La Rosa G, Kalmeijer R, Sinha R, Beumont-Mauviel M. Simeprevir with peginterferon and ribavirin leads to high rates of SVR in patients with HCV genotype 1 who relapsed after previous therapy: a phase 3 trial. *Gastroenterology* 2014; **146**: 1669-1679.e3 [PMID: 24602923 DOI: 10.1053/j.gastro.2014.02.051]
- 98 **Jacobson IM**, Dore GJ, Foster GR, Fried MW, Radu M, Rafalsky VV, Moroz L, Craxi A, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Scott J, Sinha R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a plus ribavirin in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2014; **384**: 403-413 [PMID: 24907225 DOI: 10.1016/S0140-6736(14)60494-3]
- 99 **Manns M**, Marcellin P, Poordad F, de Araujo ES, Buti M, Horsmans Y, Janczewska E, Villamil F, Scott J, Peeters M, Lenz O, Ouwerkerk-Mahadevan S, De La Rosa G, Kalmeijer R, Sinha



- R, Beumont-Mauviel M. Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2014; **384**: 414-426 [PMID: 24907224 DOI: 10.1016/S0140-6736(14)60538-9]
- 100 **European Association for the Study of the Liver.** EASL recommendations on treatment of hepatitis C 2014. *J Hepatol* 2014; **61**: 373-395 [PMID: 24818984 DOI: 10.1016/j.jhep.2014.05.001]
  - 101 **Lawitz E,** Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
  - 102 **Ruane PJ,** Ain D, Meshrekey R, Riad J, Soliman M, Mikhail S, Wolfe PR, Kersey K, Doehle B, Jiang D, Symonds WT. Sofosbuvir plus ribavirin, an interferon-free regimen, in the treatment of treatment-naïve and treatment-experienced patients with chronic genotype 4 HCV infection. *J Hepatol* 2014; **60** (Suppl 1): S503-S504
  - 103 **Jacobson IM,** Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]
  - 104 **Stepanova M,** Nader F, Cure S, Bourhis F, Hunt S, Younossi ZM. Patients' preferences and health utility assessment with SF-6D and EQ-5D in patients with chronic hepatitis C treated with sofosbuvir regimens. *Aliment Pharmacol Ther* 2014; **40**: 676-685 [PMID: 25040192 DOI: 10.1111/apt.12880]
  - 105 **Younossi ZM,** Stepanova M, Henry L, Gane E, Jacobson IM, Lawitz E, Nelson D, Nader F, Hunt S. Minimal impact of sofosbuvir and ribavirin on health related quality of life in Chronic Hepatitis C (CH-C). *J Hepatol* 2014; **60**: 741-747 [PMID: 24333184 DOI: 10.1016/j.jhep.2013.12.006]
  - 106 **Herbst DA,** Reddy KR. NS5A inhibitor, daclatasvir, for the treatment of chronic hepatitis C virus infection. *Expert Opin Investig Drugs* 2013; **22**: 1337-1346 [PMID: 23931586 DOI: 10.1517/13543784.2013.826189]
  - 107 **Hezode C,** Hirschfield GM, Ghesquiere W, Sievert W, Rodriguez-Torres M, Shafran SD, Thuluvath PJ, Tatum HA, Waked I, Esmat G, Lawitz EJ, Rustgi VK, Pol S, Weis N, Pockros PJ, Bourliere M, Serfaty L, Vierling JM, Fried MW, Weiland O, Brunetto MR, Everson GT, Zeuzem S, Kwo PY, Sulkowski M, Brau N, Hernandez D, McPhee F, Wind-Rotolo M, Liu Z, Noviello S, Hughes EA, Yin PD, Schnittman S. Daclatasvir plus peginterferon alfa and ribavirin for treatment-naïve chronic hepatitis C genotype 1 or 4 infection: a randomised study. *Gut* 2014 Jul 30; Epub ahead of print [PMID: 25080450 DOI: 10.1136/gutjnl-2014-307498]
  - 108 **Everson G,** Cooper C, Hezode C, Shiffman ML, Yoshida E, Beltran-Jaramillo T, Andreone P, Bruno S, Ferenci P, Zeuzem S, Brunda M, Le Pogam S, Najera I, Zhou J, Navarro MT, Voulgari A, Shulman NS, Yetzer ES. DAUPHINE: a randomized phase II study of danoprevir/ritonavir plus peginterferon alpha-2a/ribavirin in HCV genotypes 1 or 4. *Liver Int* 2015; **35**: 108-119 [PMID: 24517252 DOI: 10.1111/liv.12471]
  - 109 **Bronowicki JP,** Ratzu V, Gadano A, Thuluvath PJ, Bessone F, Martorell CT, Pol S, Terg R, Younes Z, He B, Eley T, Cohen D, Yu F, Hernandez D, McPhee F, Mendez P, Hughes E. Randomized trial of asunaprevir plus peginterferon alfa and ribavirin for previously untreated genotype 1 or 4 chronic hepatitis C. *J Hepatol* 2014; **61**: 1220-1227 [PMID: 25038486 DOI: 10.1016/j.jhep.2014.07.011]
  - 110 **Pockros PJ,** Jensen D, Tsai N, Taylor R, Ramji A, Cooper C, Dickson R, Tice A, Kulkarni R, Vierling JM, Lou Munson M, Chen YC, Najera I, Thommes J. JUMP-C: a randomized trial of mericitabine plus pegylated interferon alpha-2a/ribavirin for 24 weeks in treatment-naïve HCV genotype 1/4 patients. *Hepatology* 2013; **58**: 514-523 [PMID: 23359491 DOI: 10.1002/hep.26275]
  - 111 **Wedemeyer H,** Jensen D, Herring R, Ferenci P, Ma MM, Zeuzem S, Rodriguez-Torres M, Bzowej N, Pockros P, Vierling J, Ipe D, Munson ML, Chen YC, Najera I, Thommes J. PROPEL: a randomized trial of mericitabine plus peginterferon alpha-2a/ribavirin therapy in treatment-naïve HCV genotype 1/4 patients. *Hepatology* 2013; **58**: 524-537 [PMID: 23348636 DOI: 10.1002/hep.26274]

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## Highlights in pathogenesis of vitiligo

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highlight the autoimmune hypothesis, followed by the reactive oxygen species model, zinc- $\alpha$ 2-glycoprotein deficiency hypothesis, viral theory, intrinsic theory and biochemical, molecular and cellular alterations accounting for loss of functioning melanocytes in vitiligo. Many theories were elaborated to clarify vitiligo pathogenesis. It is a multifactorial disease involving the interplay of several factors. Future research is needed to clarify the interaction of these factors for better understanding of vitiligo pathogenesis and subsequent successful treatment.

**Key words:** Etiopathogenesis; Pigmentary disorder; Non-segmental vitiligo; Segmental vitiligo; Vitiligo

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**Core tip:** The pathogenesis of vitiligo elaborated by several theory. Future research needed to clarify the interaction of these factors for better understanding of vitiligo pathogenesis and subsequent successful treatment.

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## Abstract

Vitiligo is a common pigmentary disorder. Many studies across decades and all over the world have attempted to illustrate the pathogenesis behind it; however, the pathogenesis of vitiligo remains elusive. This review article, we present the findings behind the most and updated theories behind this psychologically debilitating and disfiguring disease. The discussion begun with the role of genetic predisposition followed by neural theory first proposed in the 1950s. We

## INTRODUCTION

The pathogenesis is complex and involves the interplay of multiple factors; however, the exact pathogenesis is not well known. Lerner *et al*<sup>[1]</sup> in the 1950s firstly proposed the neural theory, and after that, model of reactive oxygen species (ROS), the autoimmune hypothesis and the melanocytorrhagy hypothesis have appeared.



## DIFFERENT PATTERNS AND PHYSICAL DISTRIBUTION OF VITILIGO

Pruritus, elevated lesions, and erythematous margins present in inflammatory vitiligo. There are 2 main types: generalized vitiligo (GV) (widespread macules with a symmetrical distribution), whereas focal vitiligo (FV) (1 or few depigmented not elevated areas at a single site). After coalescing of vitiliginous areas in GV, or becomes extensive in the body with remaining of few normal areas, thus called vitiligo universalis. Non-segmental vitiligo enrolled FV and GV, but segmental vitiligo (SV) restricted to one unilateral region<sup>[2]</sup>, (Figure 1). The lesions of vitiligo are asymptomatic except in inflammatory vitiligo, which is associated with pruritus and characterized by elevated lesions, and erythematous margins.

## INHERITANCE OF VITILIGO

The inheritance is polygenic<sup>[3]</sup>. Family history exists in 6.25%-38% of patients with vitiligo<sup>[4]</sup>. Those who have recessive homozygosity at 3 epistatically interacting autosomal diallelic loci will be affected by vitiligo<sup>[5]</sup>.

### Molecular genetics-based studies

Spritz *et al*<sup>[6]</sup> (2004) revealed different loci or alleles for GV. Autoimmune susceptibility (AIS)-1, -2 (chromosome 7) and systemic lupus erythematosus vitiligo-related gene (SLEV1) (chromosome 17) both associated loci for GV and concomitant autoimmune diseases. Of 33 tested loci, only (XBP1, TSLP, and FOXP3) were primarily concomitant with GV. FOXP3 associated with X linked recessive multiple autoimmune disease syndrome. In addition, CTLA4 had association secondarily with GV, and the autoimmune diseases<sup>[7]</sup>. However, patients with GV also linked to AIS3 locus (chromosome 8)<sup>[6]</sup>.

Methylation of deoxyribonucleic acid (DNA) conducted by DNA methyltransferases (DNMT1, -3a, -3b)<sup>[8]</sup>. Monocytes in vitiliginous patients and normal volunteers showed sensitivity to alterations in methylation and revealed association between IL-10 and reactivity of autoimmune system<sup>[9,10]</sup>. In comparison with controls, methylation was increased and hypermethylation of the methylation-sensitive region in IL-10 that could alter genes expression in autoimmunity<sup>[9]</sup>. In a similar way, the role of transforming growth factor beta-receptor II (TGFB2), which inhibits the inflammatory pathways and lymphocyte activation was revealed<sup>[11,12]</sup>.

The ultraviolet radiation resistance-associated gene (UVRAG), resists photo-damage, and plays a role in autophagy<sup>[13]</sup>. In 439 controls and 225 NSV patients, UVRAG has 2 SNPs which were significantly different<sup>[14]</sup>.

Both diabetes mellitus type 1 and rheumatoid arthritis with SNPs found beside the insulin-dependent diabetes mellitus 8 locus (IDDM8)<sup>[15]</sup>. This region contains SMOC2, which enrolled in growth and development<sup>[16]</sup> and cell matrix interactions<sup>[17]</sup>.

Also, melanocyte proliferating gene 1 (MYG1), is elevated in skin of both vitiliginous patients with activity, and without activity<sup>[18]</sup>.

### Human leukocyte antigen

Studies revealed that vitiligo associated with HLA-DRB1\*07, HLA-A2, 11, 28, 31, 33, HLA-B17, 35, 40 and 44<sup>[19,20]</sup>.

Susceptibility loci of vitiligo are on chromosome 6 and in the MHC<sup>[21]</sup>. A study genotyped 6623 patients with vitiligo and 10740 controls for 34 SNPs. At 6q27, 2 SNPs found with 3 unlinked genes. These gene include RNASET2, which responsible for ribonuclease (RNase)<sup>[22]</sup>. The other two genes are the chemokine receptor 6 gene (CCR6)<sup>[21]</sup>, and FGFR10P are imperative to progressing of the cycle of the cell that produce receptor of (FGF)<sup>[23]</sup>. Genes encode discoidin domain receptor 1 (DDR1)<sup>[24]</sup>, and tyrosine kinase receptor play role in cell's progression and function<sup>[25]</sup>, both were involved in vitiligo.

## THEORIES FOR VITILIGO PATHOGENESIS

### The neural theory

**Early theories:** The "neural theory" supposed by Lerner's (1959) was based on the fact that SV follows the course of the dermatome with exhibiting hyperhidrosis and emotional upset<sup>[1,26,27]</sup>.

### The sympathetic nervous system's role:

Dysfunction of sympathetic nervous system's role (SNS) activity affect melanin production and lead to depigmentation. With iontophoresis and laser Doppler flowmetry level of microcirculation in lesions with vitiligo assessed to reveal SNS activity<sup>[28]</sup>. 10 subjects had facial SV, and 2 groups of controls were examined. 1 control group had 10 healthy, unaffected individuals, and the 2<sup>nd</sup> control group contained 10 non-segmental-type stable vitiligo patients. Patients were matched for gender and age. Approximately, the cutaneous blood flow was higher three times on the lesions vs normal skin in SV. The differences was not revealed in the non-SV.

**Neuropeptide and neuronal markers:** Neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), and polyclonal general neuronal marker (PGP) tested for their immuno-reactivity in 12 patients with vitiligo and 7 unaffected control subjects<sup>[29]</sup>. NPY increased in the marginal areas of lesions in half of the patients vs normal, and associated with noradrenaline with exerting a local autonomic effect<sup>[29]</sup>. Lazarova *et al*<sup>[30]</sup> (2000) confirmed this finding; however, they found that CGRP was also non-significantly increased in vitiligo. Precipitating factor, as, stress, produce significant level of neuropeptides such as NPY that induce the disease<sup>[30,31]</sup>. A cohort study revealed

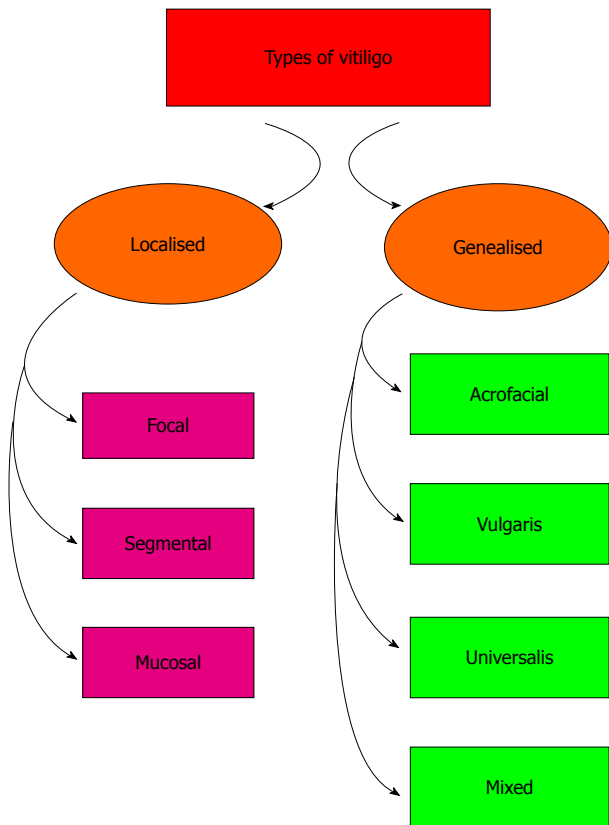


Figure 1 Types of vitiligo.

increased levels of nerve growth factor (NGF) significantly in vitiligo<sup>[32]</sup>. Stress up regulates NGF expression in hair follicles, decreases the high affinity TrkA receptor, increases production of p75NTR NGF-receptor, and increases in dorsal root ganglia the substance P neurons<sup>[33]</sup>.

Catecholamine metabolite levels [homovanillic acid (HVA), vanilmandelic acid (VMA), 3-methoxytyramine (MT), normetanephrine (NMN), metanephrine (MN), 3,4-dihydroxy mandelic acid (DOMAC), and 3,4-dihydroxy phenylacetic acid (DOPAC)] were measured in 1-d urinary samples of 150 vitiliginous subjects and 50 normal subjects. HVA and VMA levels corresponded to the activity of the disease<sup>[34]</sup>. Stressors result in catecholamines discharge, which bind  $\alpha$ -R in the mucosa and skin arteriolar wall leading to vasoconstriction, hypoxia, and overproduction of oxygen radicals that destroy melanocytes<sup>[34]</sup>. Mental stress could stimulate the hypothalamic-pituitary-adrenal axis and then secretion of catecholamines<sup>[34,35]</sup>.

### The autoimmune hypothesis

The etio-pathogenesis of "generalized" or non-segmental vitiligo is better explained by autoimmune mechanisms as vitiligo often has autoimmune comorbidities and it often responds to immunosuppressive treatments<sup>[36]</sup>. The reaction of immunity are cell-mediated, humoral (antibody-mediated), or through the cytokines.

**The role of humoral immunity:** In 2010, tyrosine

hydroxylase antibodies checked with radioimmunoassay (RIA) in sera were obtained from 79 non-SV patients, 8 patients with SV, 91 subjects with other autoimmune diseases and 28 healthy subjects. TH antibodies revealed significantly in non-SV. Also, in non-SV, antibodies against MCHR1 (melanin-concentrating hormone receptor 1), tyrosinase<sup>[37]</sup> and pigment cell-surface antigens<sup>[38]</sup> were noted.

In 80% of active vitiligo patients, immunoglobulin G (IgG) and immunoglobulin M (IgM) against melanocytes were found. Low levels IgA also found in the inactive and control groups<sup>[38]</sup>.

Furthermore, anti-thyroglobulin antibodies, antithyroid antibodies, anti-thyroperoxidase, and antismooth muscle antibody are present. Those are typically related to thyroid disease and other autoimmune diseases<sup>[39,40]</sup>.

Melanin concentrating hormone (MCH) binds MCHR1 thus increase calcium influx and acting as an antagonist of  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH)<sup>[41-43]</sup>.

**The role of cell-mediated immunity:** Immunohistochemical examination of the inflammatory infiltrates in perilesional vitiligo skin using single and double immunostaining for melanocytes, Langerhans cells, T-cells, and macrophages revealed higher densities of melanocytes in normal skin, vs non-affected skin in subjects with vitiligo. These T cell had dramatic production of (IL-2R), and increased CD8:CD4 ratio. Thus, melanocytes destruction may be cytotoxic CD8 T-cell mediated. Perilesional HLA-DR production (MHC class II receptor) exhibited in all of the patients with vitiligo, especially along suprabasal and basal keratinocytes, due to local T cell reactivity. In addition, macrophages were numerous in vitiligo vs controls, whereas the CD36 subset of macrophages were higher in the later<sup>[44]</sup>.

**The role of cytokines in vitiligo:** Beyond lymphocytes and antibodies, the immune system has a complex interplay of many cytokines. There are significantly increased expression of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferon- $\gamma$  (IFN- $\gamma$ ), and IL-10<sup>[45]</sup>. As IFN- $\gamma$  and TNF- $\alpha$  are T helper cell-1 (Th1) cytokines, so vitiligo is mediated by the Th1 response<sup>[46]</sup>.

IL-17 plays role with macrophages, keratinocytes, and fibroblasts. In addition, it activates the expression of others, as IL-1 and IL-6, and TNF- $\alpha$ <sup>[47,48]</sup>. Examination of sera and tissue of 30 vitiliginous subjects and 20 normal subjects showed significant higher levels of IL-17 toward vitiliginous subjects and disease duration<sup>[47]</sup>.

**The biochemical Theory- reactive oxygen species model** Oxidative stress hypothesis suggests that imbalanced redox (reduction-oxidation) state of the vitiliginous skin. This results in the dramatic production of reactive oxygen species (ROS), as H<sub>2</sub>O<sub>2</sub>. ROS oxidize components of the cell leading to melanocytes destruction and creating the depigmented macules<sup>[49]</sup>.

**The redox status of vitiligo patients:** Sera of

thirty-six patients with vitiligo (18 with inactive and 18 with unstable disease), and 40 normal subjects were examined for main factors of redox status involving selenium, malondialdehyde (MDA), vitamins A and E, and glutathione peroxidase (GPx) in the erythrocyte activities, catalase (CAT) and superoxide dismutase (SOD). Superoxide radicals are scavenged and their toxicity is reduced with SOD which transforms  $O_2^-$  to  $H_2O_2$  and  $O_2$ , and catalase transforms ( $H_2O_2$ ) to ( $H_2O$ ) and ( $O_2$ )<sup>[50]</sup>. As MDA results from lipid peroxidation, it is a marker of oxidative stress<sup>[51,52]</sup>. Selenium is required for GPx activity and vitamins A and E are important in antioxidant activity. Serum selenium, SOD and MDA are prominent in both unstable and inactive types. By enhancing SOD activity, the  $H_2O_2$  accumulates. In addition, GPx detoxifies  $H_2O_2$  (downstream enzyme). Therefore, GPx levels decreased in patients with vitiligo<sup>[50,53]</sup>.

Increased SOD activity in patients with vitiligo is a response to oxidative stress; thus,  $H_2O_2$  elevate as could not be eradicated by low level of CAT<sup>[53,54]</sup>.

### The role of tetrahydrobiopterin recycling in vitiligo:

Another cellular pathway affected by accumulating  $H_2O_2$  involves tetrahydrobiopterin. Tyrosinase is an imperative enzyme in formation of melanin<sup>[55]</sup>. L-tyrosine synthased from L-phenylalanine by the phenylalanine hydroxylase (PAH). The 5, 6, 7, 8-tetrahydrobiopterin or 6BH4 is essential cofactor for this process is. Defective recycling of 6BH4 lead to excess 7BH4 that is an inhibitor of PAH. Uncoupled PAH and 7BH4 found in suction blister material from the skin of vitiligo patients<sup>[56]</sup>. Kowlessur *et al*<sup>[57]</sup> (1996) also found that 7BH4 production yields  $H_2O_2$ . Haase *et al*<sup>[58]</sup> (2004) studied the enzyme dihydropteridine reductase (DHPR) (imperative to end the normal 6BH4 recycling). They assessed whole blood samples from 27 untreated vitiligo subjects and 8 normal subjects. The results showed that DHPR activity decrease with high concentrations of  $H_2O_2$  and *vice versa*.

Effect of  $H_2O_2$  on acetylcholinesterase (AChE) decrease in patients with vitiligo vs healthy controls<sup>[59,60]</sup>. Thus, AChE dependent on  $H_2O_2$  concentration levels, *i.e.*, low  $H_2O_2$  concentrations (approximately 10-6M or mol/L) activate AChE whereas high concentrations (10-3M or mol/L) deactivate AChE<sup>[60]</sup>. Butyrylcholinesterase (BchE) mediates the hydrolysis of acetylcholine. The hydrolysis reaction is one of the rate-limiting steps in cholinergic signal transduction<sup>[61,62]</sup>.

In 2008, showed that xanthine oxidase (XO) is a source of  $H_2O_2$ . High concentrations of  $H_2O_2$  inhibit the activity of XO, and *vice versa*<sup>[63]</sup>.

Briefly, there are at least 5 important pathways enrolled in  $H_2O_2$  overproduction in vitiligo: (1) Defective recycling of 6BH4<sup>[56,64,65]</sup>; (2) Catecholamine formation increased as levels of monoamine oxidase A (MAO) increased<sup>[34,66,67]</sup>; (3) Inhibition of thioredoxin/thioredoxin reductase by calcium<sup>[68-70]</sup>; (4) NADPH oxidase activities

increased by the cellular infiltrate<sup>[71]</sup>; and (5) Nitric oxide synthase (NOS) activities increased<sup>[71]</sup>.

Oxidative stress affects calcium homeostasis at the cellular level<sup>[72]</sup> in melanocytes and keratinocytes in vitiliginous patients<sup>[69]</sup>.

### Zinc- $\alpha$ 2-Glycoprotein deficiency hypothesis

For the first time, Bagherani *et al*<sup>[73]</sup> and Yaghoobi *et al*<sup>[74]</sup> pointed the probable association which might be present between ZAG and vitiligo<sup>[73,74]</sup>. It was suggested that the pathogenesis of vitiligo could be attributed to decrease in ZAG as follows: (1) Studies have demonstrated that ZAG is acting as a keratinocyte-derived factor influencing melanocyte proliferation and dendricity<sup>[75,76]</sup>. So, ZAG could be considered as a marker of cells differentiation and maturation<sup>[76]</sup>; (2) A chronic detachment of melanocytes is an imperative pillar in the pathogenesis of vitiligo<sup>[74,77,78]</sup>. Thus, melanocyte adhesions to the other cells in epidermis will be impaired in the lack of ZAG; (3) Topical steroids are the most safeand effective forms of treatment for vitiligo, especially for the localized one<sup>[74,79]</sup>, because of their ability to increase ZAG expression<sup>[80,81]</sup>; (4) Some studies have shown that zinc can precipitate ZAG<sup>[74,82]</sup>. Thus, the effectivity of zinc in treating this disease is related to its ability to precipitate circulating ZAG at the site of vitiligo<sup>[73]</sup>; and (5) The linkage signals on chromosome 7 in patients with GV and associated autoimmune diseases have been reported<sup>[73,83]</sup>. Surprisingly, ZAG gene is located on the chromosome 7<sup>[76]</sup>.

### Viral theory

There is a strong association between vitiligo and chronic hepatitis C virus (HCV) infection and autoimmune hepatitis<sup>[84]</sup>. Akcan *et al*<sup>[85]</sup> in 2006 reported a low hepatitis B virus (HBV) sero-positivity in vitiliginous patients. Previous or concurrent cytomegalovirus (CMV) infections may induce the etio-pathogenesis or deterioration of vitiligo<sup>[85,86]</sup>.

Furthermore, other viruses as Epstein-Barr virus, hepatitis E virus, herpes virus and the human immunodeficiency virus (HIV) also have suspicious association with vitiligo<sup>[86,87]</sup>.

### Intrinsic theory

Melanocytes in vitiligo have an intrinsic defect leading to their death. They demonstrate different abnormalities, including abnormal rough endoplasmic reticulum or deficiency of unidentified melanocyte growth factors such as basic fibroblast growth factor (bFGF) and decrease in the number of melanocytes expressing the c-kit receptor in lesional skin<sup>[88,89]</sup>.

Melanocytes require a constant keratinocyte-derived c-Kit stimulation for their maintenance<sup>[90]</sup>, thus weak expression of keratinocyte-derived factors, as stem cell factor (SCF), may lead to passive melanocyte death and might explain the Koebner phenomenon<sup>[91]</sup>.

### Cellular, molecular and biochemical alterations and melanocytes loss of in vitiligo

Recently, malfunctioning melanocytes found in vitiliginous lesions<sup>[92]</sup>. Electron microscopy, reverse transcription PCR (polymerase chain reaction) and southern blotting experiments revealed sporadic survival of melanocyte in vitiliginous lesions<sup>[93,94]</sup>. Thus, this points to presence of immature melanocyte precursors/stem cells<sup>[95,96]</sup>. Now, 2 pathways have been supposed for melanocytes loss: highly programmed death by apoptosis<sup>[77,91,97,98]</sup> and accelerated cell senescence<sup>[99]</sup>.

### Apoptosis and accelerated cell senescence:

Melanocytes from non-lesional skin of vitiligo patients have abnormalities as cytoplasm vacuolization, rough endoplasmic reticulum dilatation, DNA marginalization in the nucleus, loss of dendrites and detachment<sup>[88,99,100]</sup>.

Regarding keratinocytes, apoptosis occurs at least in the traumatized vitiligo skin<sup>[101]</sup>. Thus, basal and suprabasal epidermal cells in the depigmented and normally pigmented skin show degeneration due to swelling of the membrane-bound organelles, formation of vacuoles and cytoplasm condensation<sup>[102]</sup>.

As vitiligo could induced by trauma (Koebner's phenomenon). Lee *et al*<sup>[97]</sup> revealed the lower expression levels of the antiapoptotic Bcl-2 and FLIP proteins in vitiliginous skin vs the normally pigmented skin. On the other hand, there was dramatic levels of the proapoptotic Bax and p53 proteins and of the active forms of caspase-3, 8 and 9<sup>[97]</sup>.

Apoptosis triggered by normal developmental program, UV light, H<sub>2</sub>O<sub>2</sub>, staurosporine and other stimuli<sup>[91,103]</sup>. *NALP1* gene that stimulates cellular apoptosis<sup>[102,104]</sup>, is associated with vitiligo susceptibility<sup>[105,106]</sup>.

Epidermal melanocytes from epidermal melanin unit produce growth factors (GF) for melanocytes<sup>[107]</sup>. Therefore, its damage have imperative effects on melanocyte survival<sup>[91]</sup>. Thus, low levels of GF as SCF, endothelin-1 (ET-1) or high levels of melanocyte inhibiting cytokines, as TNF- $\alpha$  and IL-6 may lead to keratinocyte apoptosis, and then apoptosis of melanocytes<sup>[108]</sup>.

Life span of lesional keratinocytes is greatly shortened when compared to the life span of normal and non-lesional vitiligo keratinocytes. It also shows modification of proliferation and senescence marker expression (p16, p53, p21), when compared to keratinocytes from clinically noninvolved skin<sup>[98]</sup>.

Although apoptosis and senescence of epidermal keratinocytes is a response to various stimuli, they also share some cellular mechanisms and controlled by similar molecular regulators<sup>[109]</sup>. Both apoptosis and aging induced by stress signals as ROS accumulation and DNA damage<sup>[110]</sup>. The paradigmatic proapoptotic factor p53<sup>[111]</sup>, is also a guard keeper of DNA integrity which triggers cell cycle arrest in DNA damaged cells<sup>[109]</sup>.

**Melanocytorrhagy theory:** Gauthier *et al*<sup>[78]</sup> in 2003 mentioned that NSV occurs due to "melanocytorrhagy", or a chronic melanocytes detachment and loss caused by trauma and other stressors include catecholamines, ROS, or autoimmune elements. This theory combined the concepts from other theories mentioned before to elaborate a single integrated explanation of vitiligo pathogenesis<sup>[78]</sup>.

A study done by Tobin *et al*<sup>[92]</sup> in 2000 proposed loss of melanocytes in vitiligo. They explained these findings because of oxidative stress caused by H<sub>2</sub>O<sub>2</sub>. Gauthier *et al*<sup>[78]</sup> (2003) also reported that impaired cell adhesion plays a role in vitiligo pathogenesis as the synthesis of extracellular matrix components by keratinocytes may be defective, the presence of focal gaps in the basement membrane and impaired formation of basement membrane. These abnormalities weaken the basal attachment of melanocytes. Trauma could aggravate this susceptibility with subsequent chronic melanocyte loss, known as melanocytorrhagy.

Le Poole *et al*<sup>[112]</sup> mentioned that the protein tenascin may play a role in decreasing melanocytes adhesion in vitiligo. This protein was highly expressed in patients with vitiligo than the controls<sup>[112]</sup>. This can explain the development of vitiligo by Koebner phenomenon, which represent "transepidermal migration"<sup>[78,113]</sup>.

### Integrated theory (Conversion theory)

Despite all the mentioned theories are attractive, it is likely that vitiligo is a result of the convergence of these pathological pathways. Most experts agree that vitiligo may be a syndrome with a multifactorial etiology rather than a single entity<sup>[114]</sup> (Figure 2).

## TREATMENT

The disfigurement associated with vitiligo could cause serious emotional stress for the patient and affect his quality of life<sup>[115]</sup>. Sun protection of vitiliginous areas with sun blocks is imperative<sup>[115,116]</sup> to prevent sunburn, photodamage and occurrence of Koebner phenomenon. In addition, sun blocks decrease tanning of the uninvolved skin and thus lessen the contrast with vitiliginous lesions<sup>[117]</sup>.

There is no treatment ensures complete cure of vitiligo. Therefore, there is a plethora of modalities, such as topical corticosteroids, vitamin-D derivatives, calcineurin inhibitors, photochemotherapy [psoralen plus UV-A (PUVA), psoralen with sunlight (PUVAso)], phototherapy (UV-A, narrowband UV-B), surgical techniques<sup>[117-122]</sup>, excimer laser<sup>[115,117,118-123]</sup>, topical prostaglandin E (PGE2)<sup>[118]</sup>, and combinations of topical therapies and light treatment<sup>[79]</sup>. Complementary and integrative therapies are also used, as ginkgo biloba<sup>[79]</sup>, and levamisole<sup>[124]</sup>, because of their immune-modulating properties<sup>[117]</sup>.

Pseudocatalase cream with Dead Sea climatotherapy can also promote repigmentation<sup>[117]</sup>. Topical flu-



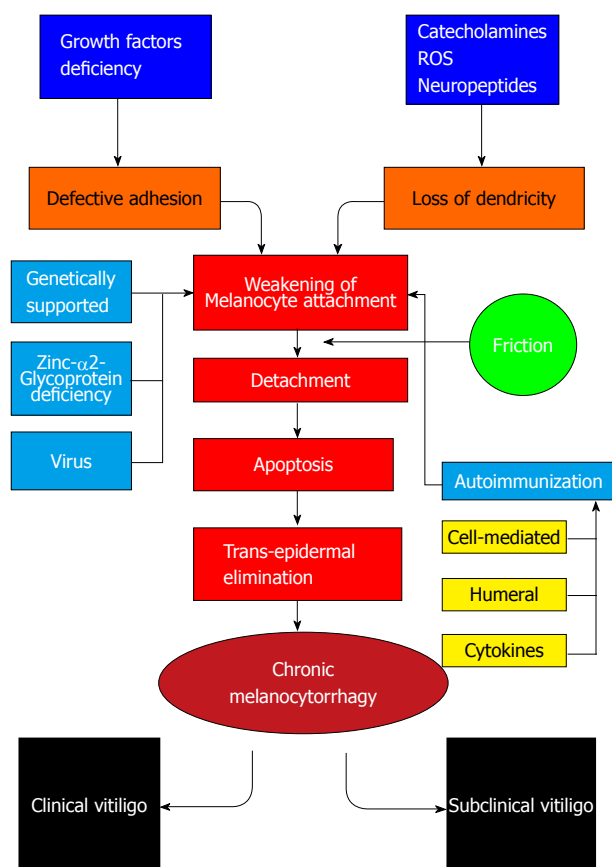


Figure 2 Pathogenesis of vitiligo.

orouracil<sup>[125]</sup>, topical melagenina I and II, minoxidil<sup>[118]</sup>, oral L-phenylalanine<sup>[117,126-129]</sup>, homeopathy, ayurvedic medicine, clintologic and balneologic therapies are other therapeutic options for vitiligo<sup>[118]</sup>. Patients with widespread disease (affecting more than half of body) seeking stable matching of skin color but for whom repigmentation is not expected will be more satisfied if normal pigmented areas are depigmented with 20% monobenzyl ether of hydroquinone, twice per day for almost one year. Another helpful modality for vitiligo universalis, is combined both the topical application of 4-methoxyphenol and the Q-switched (QS) ruby laser<sup>[115]</sup>. Q-Switched ruby laser destroy the melanosomes present in melanocytes and keratinocytes by selective photothermolysis<sup>[130]</sup>.

According to the impact of oxidative stress on vitiligo,  $\alpha$ -tocopherol can be used alone or with topical corticosteroids in combination with psoralen plus ultraviolet A (PUVA)<sup>[131,132]</sup>. Antioxidant pool (tocopherol acetate, ubiquinone, selenomethionine, methionine) could be used in vitiligo aiming to enhance the enzymatic and the non-enzymatic antioxidant activity<sup>[131,132]</sup>. Food additives, contaminants, preservatives and cosmetic products could exacerbate vitiligo due to oxidative stress in melanocytes<sup>[133]</sup>.

High consumption of omega-6 and decreased omega-3 intake produce free radicals and pro-inflammatory cytokines. On the other hand, omega-3 intake exert protection by enhancing TGF- $\beta$  mRNA

levels and antioxidant enzymes<sup>[134]</sup> and inhibiting pro-inflammatory cytokines as TNF- $\alpha$ <sup>[135]</sup>.

Cell membranes enriched with omega-3 polyunsaturated fatty acids have elevated activity of glutathione peroxidase (GSH)<sup>[136]</sup>. In addition, omega-3 fatty acids contains indole-3-carbinol which activates CYP1A1 that is responsible for hydroxylation of estrogens to 2-hydroxyestrene<sup>[137]</sup>. Furthermore, omega-3 fatty acids have a vital role in the function of the central nervous system and affect the susceptibility and prognosis of depression<sup>[135]</sup>. Twenty percent of patients with vitiligo are found to have depression. This highlights the benefits of these lipids in vitiligo<sup>[137]</sup>.

In conclusion, many theories were elaborated to clarify vitiligo pathogenesis. It is a multifactorial disease involving the interplay of several factors. Future research is needed to clarify the interaction of these factors for better understanding of vitiligo pathogenesis and subsequent successful treatment.

## REFERENCES

1. Lerner AB. Vitiligo. *J Invest Dermatol* 1959; **32**: 285-310 [PMID: 13641799 DOI: 10.1038/jid.1959.49]
2. Wolff K, Johnson RA, Suurmond D. Section 13: Pigmentary Disorders-VITILIGO. In: Fitzpatrick's Color Atlas & Synopsis of Clinical Dermatology, 6th ed. New York: McGraw-Hill, 2009 Available from: URL: <http://www.accessmedicine.com/login.ezproxy.library.ualberta.ca/content.aspx?aID=5187746>
3. Njoo MD, Westerhof W. Vitiligo. Pathogenesis and treatment. *Am J Clin Dermatol* 2001; **2**: 167-181 [PMID: 11705094 DOI: 10.2165/00128071-200102030-00006]
4. Nath SK, Majumder PP, Nordlund JJ. Genetic epidemiology of vitiligo: multilocus recessivity cross-validated. *Am J Hum Genet* 1994; **55**: 981-990 [PMID: 7977362]
5. Kim SM, Chung HS, Hann SK. The genetics of vitiligo in Korean patients. *Int J Dermatol* 1998; **37**: 908-910 [PMID: 9888330 DOI: 10.1046/j.1365-4362.1998.00549.x]
6. Spritz RA, Gowan K, Bennett DC, Fain PR. Novel vitiligo susceptibility loci on chromosomes 7 (AIS2) and 8 (AIS3), confirmation of SLEVI on chromosome 17, and their roles in an autoimmune diathesis. *Am J Hum Genet* 2004; **74**: 188-191 [PMID: 14691733 DOI: 10.1086/381134]
7. Birlea SA, Jin Y, Bennett DC, Herbstman DM, Wallace MR, McCormack WT, Kemp EH, Gawkrödger DJ, Weetman AP, Picardo M, Leone G, Taïeb A, Jouary T, Ezzedine K, van Geel N, Lambert J, Overbeck A, Fain PR, Spritz RA. Comprehensive association analysis of candidate genes for generalized vitiligo supports XBP1, FOXP3, and TSLP. *J Invest Dermatol* 2011; **131**: 371-381 [PMID: 21085187 DOI: 10.1038/jid.2010.337]
8. Li E. Chromatin modification and epigenetic reprogramming in mammalian development. *Nat Rev Genet* 2002; **3**: 662-673 [PMID: 12209141]
9. Zhao M, Gao F, Wu X, Tang J, Lu Q. Abnormal DNA methylation in peripheral blood mononuclear cells from patients with vitiligo. *Br J Dermatol* 2010; **163**: 736-742 [PMID: 20560952 DOI: 10.1111/j.1365-2133.2010.09919.x]
10. Szalmás A, Bánáti F, Koroknai A, László B, Fehér E, Salamon D, Gergely L, Minárovits J, Kónya J. Lineage-specific silencing of human IL-10 gene expression by promoter methylation in cervical cancer cells. *Eur J Cancer* 2008; **44**: 1030-1038 [PMID: 18378443]
11. Yun JY, Uhm YK, Kim HJ, Lim SH, Chung JH, Shin MK, Yim SV, Lee MH. Transforming growth factor beta receptor II (TGFBR2) polymorphisms and the association with nonsegmental vitiligo in the Korean population. *Int J Immunogenet* 2010; **37**: 289-291



- [PMID: 20518838 DOI: 10.1111/j.1744-313X.2010.00923.x]
- 12 **Basak PY**, Adiloglu AK, Ceyhan AM, Tas T, Akkaya VB. The role of helper and regulatory T cells in the pathogenesis of vitiligo. *J Am Acad Dermatol* 2009; **60**: 256-260 [PMID: 19022528]
  - 13 **Liang C**, Feng P, Ku B, Dotan I, Canaani D, Oh BH, Jung JU. Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat Cell Biol* 2006; **8**: 688-699 [PMID: 16799551 DOI: 10.1038/ncb1426]
  - 14 **Jeong TJ**, Shin MK, Uhm YK, Kim HJ, Chung JH, Lee MH. Association of UVRAG polymorphisms with susceptibility to non-segmental vitiligo in a Korean sample. *Exp Dermatol* 2010; **19**: e323-e325 [PMID: 20163458 DOI: 10.1111/j.1600-0625.2009.01039.x]
  - 15 **Birlea SA**, Gowan K, Fain PR, Spritz RA. Genome-wide association study of generalized vitiligo in an isolated European founder population identifies SMOC2, in close proximity to IDDM8. *J Invest Dermatol* 2010; **130**: 798-803 [PMID: 19890347 DOI: 10.1038/jid.2009.347]
  - 16 **Liu P**, Pazin DE, Merson RR, Albrecht KH, Vaziri C. The developmentally-regulated Smoc2 gene is repressed by Aryl-hydrocarbon receptor (Ahr) signaling. *Gene* 2009; **433**: 72-80 [PMID: 19146932 DOI: 10.1016/j.gene.2008.12.010]
  - 17 **Maier S**, Paulsson M, Hartmann U. The widely expressed extracellular matrix protein SMOC-2 promotes keratinocyte attachment and migration. *Exp Cell Res* 2008; **314**: 2477-2487 [PMID: 18582461]
  - 18 **Kingo K**, Philips MA, Aunin E, Luuk H, Karelson M, Rätsep R, Silm H, Vasar E, Kõks S. MYG1, novel melanocyte related gene, has elevated expression in vitiligo. *J Dermatol Sci* 2006; **44**: 119-122 [PMID: 16996721]
  - 19 **Hu DY**, Ren YQ, Zhu KJ, Lv YM, Cheng H, Zhang Z, Li Y, He SM, Tang J, Liu JL, Lin Y, Sun YY, Zuo XB, Chen G, Sun LD, Yang S, Zhang XJ. Comparisons of clinical features of HLA-DRB1\*07 positive and negative vitiligo patients in Chinese Han population. *J Eur Acad Dermatol Venereol* 2011; **25**: 1299-1303 [PMID: 21241376 DOI: 10.1111/j.1468-3083.2010.03971.x]
  - 20 **Misri R**, Khopkar U, Shankarkumar U, Ghosh K. Comparative case control study of clinical features and human leukocyte antigen susceptibility between familial and nonfamilial vitiligo. *Indian J Dermatol Venereol Leprol* 2009; **75**: 583-587 [PMID: 19915238 DOI: 10.4103/0378-6323.57719]
  - 21 **Quan C**, Ren YQ, Xiang LH, Sun LD, Xu AE, Gao XH, Chen HD, Pu XM, Wu RN, Liang CZ, Li JB, Gao TW, Zhang JZ, Wang XL, Wang J, Yang RY, Liang L, Yu JB, Zuo XB, Zhang SQ, Zhang SM, Chen G, Zheng XD, Li P, Zhu J, Li YW, Wei XD, Hong WS, Ye Y, Zhang Y, Wu WS, Cheng H, Dong PL, Hu DY, Li Y, Li M, Zhang X, Tang HY, Tang XF, Xu SX, He SM, Lv YM, Shen M, Jiang HQ, Wang Y, Li K, Kang XJ, Liu YQ, Sun L, Liu ZF, Xie SQ, Zhu CY, Xu Q, Gao JP, Hu WL, Ni C, Pan TM, Li Y, Yao S, He CF, Liu YS, Yu ZY, Yin XY, Zhang FY, Yang S, Zhou Y, Zhang XJ. Genome-wide association study for vitiligo identifies susceptibility loci at 6q27 and the MHC. *Nat Genet* 2010; **42**: 614-618 [PMID: 20526339 DOI: 10.1038/ng.603]
  - 22 **Thompson DM**, Parker R. The RNase Rny1p cleaves tRNAs and promotes cell death during oxidative stress in *Saccharomyces cerevisiae*. *J Cell Biol* 2009; **185**: 43-50 [PMID: 19332891 DOI: 10.1083/jcb.200811119]
  - 23 **Acquaviva C**, Chevrier V, Chauvin JP, Fournier G, Birnbaum D, Rosnet O. The centrosomal FOP protein is required for cell cycle progression and survival. *Cell Cycle* 2009; **8**: 1217-1227 [PMID: 19305129 DOI: 10.4161/cc.8.8.8248]
  - 24 **Silva de Castro CC**, do Nascimento LM, Walker G, Werneck RI, Nogoceke E, Mira MT. Genetic variants of the DDR1 gene are associated with vitiligo in two independent Brazilian population samples. *J Invest Dermatol* 2010; **130**: 1813-1818 [PMID: 20182441 DOI: 10.1038/jid.2010.34]
  - 25 **Yoshimura T**, Matsuyama W, Kamohara H. Discoidin domain receptor 1: a new class of receptor regulating leukocyte-collagen interaction. *Immunol Res* 2005; **31**: 219-230 [PMID: 15888913 DOI: 10.1385/IR.31:3:219]
  - 26 **Koga M**, Tango T. Clinical features and course of type A and type B vitiligo. *Br J Dermatol* 1988; **118**: 223-228 [PMID: 3348967 DOI: 10.1111/j.1365-2133.1988.tb01778.x]
  - 27 **Manolache L**, Banea V. Stress in patients with alopecia areata and vitiligo. *J Eur Acad Dermatol Venereol* 2007; **21**: 921-928 [PMID: 17659001 DOI: 10.1111/j.1468-3083.2006.02106.x]
  - 28 **Wu CS**, Yu HS, Chang HR, Yu CL, Yu CL, Wu BN. Cutaneous blood flow and adrenoceptor response increase in segmental-type vitiligo lesions. *J Dermatol Sci* 2000; **23**: 53-62 [PMID: 10699765 DOI: 10.1016/S0923-1811(99)00090-0]
  - 29 **Al'Abadie MS**, Senior HJ, Bleehen SS, Gawkrödger DJ. Neuropeptide and neuronal marker studies in vitiligo. *Br J Dermatol* 1994; **131**: 160-165 [PMID: 7522512]
  - 30 **Lazarova R**, Hristakieva E, Lazarov N, Shani J. Vitiligo-related neuropeptides in nerve fibers of the skin. *Arch Physiol Biochem* 2000; **108**: 262-267 [PMID: 11094379 DOI: 10.1076/1381-3455(200007)108:3;1-Z;FT262]
  - 31 **Yehuda R**, Brand S, Yang RK. Plasma neuropeptide Y concentrations in combat exposed veterans: relationship to trauma exposure, recovery from PTSD, and coping. *Biol Psychiatry* 2006; **59**: 660-663 [PMID: 16325152 DOI: 10.1016/j.biopsych.2005.08.027]
  - 32 **Rateb AAH**, Azzam OA, Rashed LA, El-Guindy NM, El-Din MS. The role of nerve growth factor in the pathogenesis of vitiligo. *JEWDS* 2005; **1**: 18-24. Available from: URL: <http://www.jewds.eg.net/pdf/2004/w1a1a3.PDF>
  - 33 **Peters EM**, Handjiski B, Kuhlmei A, Hagen E, Bielas H, Braun A, Klapp BF, Paus R, Arck PC. Neurogenic inflammation in stress-induced termination of murine hair growth is promoted by nerve growth factor. *Am J Pathol* 2004; **165**: 259-271 [PMID: 15215181 DOI: 10.1016/S0002-9440(10)63294-4]
  - 34 **Morrone A**, Picardo M, de Luca C, Terminali O, Passi S, Ippolito F. Catecholamines and vitiligo. *Pigment Cell Res* 1992; **5**: 65-69 [PMID: 1321419 DOI: 10.1111/j.1600-0749.1992.tb00003.x]
  - 35 **Stokes PE**, Sikes CR. The hypothalamic-pituitary-adrenocortical axis in major depression. *Neurol Clin* 1988; **6**: 1-19 [PMID: 2837631]
  - 36 **Lepe V**, Moncada B, Castaneda-Cazares JP, Torres-Alvarez MB, Ortiz CA, Torres-Rubalcava AB. A double-blind randomized trial of 0.1% tacrolimus vs 0.05% clobetasol for the treatment of childhood vitiligo. *Arch Dermatol* 2003; **139**: 581-585 [PMID: 12756094 DOI: 10.1001/archderm.139.5.581]
  - 37 **Kemp EH**, Emhemad S, Akhtar S, Watson PF, Gawkrödger DJ, Weetman AP. Autoantibodies against tyrosine hydroxylase in patients with non-segmental (generalised) vitiligo. *Exp Dermatol* 2011; **20**: 35-40 [PMID: 21158937 DOI: 10.1111/j.1600-0625.2010.01181.x]
  - 38 **Harning R**, Cui J, Bystryn JC. Relation between the incidence and level of pigment cell antibodies and disease activity in vitiligo. *J Invest Dermatol* 1991; **97**: 1078-1080 [PMID: 1748818 DOI: 10.1111/1523-1747.ep12492607]
  - 39 **Ingordo V**, Gentile C, Iannazzone SS, Cusano F, Naldi L. Vitiligo and autoimmunity: an epidemiological study in a representative sample of young Italian males. *J Eur Acad Dermatol Venereol* 2011; **25**: 105-109 [PMID: 20477923 DOI: 10.1111/j.1468-3083.2010.03696.x]
  - 40 **Uncu S**, Yaylı S, Bahadır S, Oktan A, Alpaly K. Relevance of autoimmune thyroiditis in children and adolescents with vitiligo. *Int J Dermatol* 2011; **50**: 175-179 [PMID: 21244381 DOI: 10.1111/j.1365-4632.2010.04665.x]
  - 41 **Chambers J**, Ames RS, Bergsma D, Muir A, Fitzgerald LR, Hervieu G, Dytko GM, Foley JJ, Martin J, Liu WS, Park J, Ellis C, Ganguly S, Konchar S, Cluderay J, Leslie R, Wilson S, Sarau HM. Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1. *Nature* 1999; **400**: 261-265 [PMID: 10421367 DOI: 10.1038/22313]
  - 42 **Kemp EH**, Waterman EA, Hawes BE, O'Neill K, Gottumukkala RV, Gawkrödger DJ, Weetman AP, Watson PF. The melanin-concentrating hormone receptor 1, a novel target of autoantibody responses in vitiligo. *J Clin Invest* 2002; **109**: 923-930 [PMID:

- 11927619 DOI: 10.1172/JCI14643]
- 43 **Ancans J**, Hoogduijn MJ, Thody AJ. Melanosomal pH, pink locus protein and their roles in melanogenesis. *J Invest Dermatol* 2001; **117**: 158-159 [PMID: 11442766 DOI: 10.1046/j.0022-202x.2001.01397.x]
- 44 **Le Poole IC**, van den Wijngaard RM, Westerhof W, Das PK. Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. *Am J Pathol* 1996; **148**: 1219-1228 [PMID: 8644862]
- 45 **Grimes PE**, Morris R, Avaniss-Aghajani E, Soriano T, Meraz M, Metzger A. Topical tacrolimus therapy for vitiligo: therapeutic responses and skin messenger RNA expression of proinflammatory cytokines. *J Am Acad Dermatol* 2004; **51**: 52-61 [PMID: 15243524 DOI: 10.1016/j.jaad.2003.12.031]
- 46 **Taher ZA**, Lauzon G, Maguiness S, Dytoc MT. Analysis of interleukin-10 levels in lesions of vitiligo following treatment with topical tacrolimus. *Br J Dermatol* 2009; **161**: 654-659 [PMID: 19438859 DOI: 10.1111/j.1365-2133.2009.09217.x]
- 47 **Bassiouny DA**, Shaker O. Role of interleukin-17 in the pathogenesis of vitiligo. *Clin Exp Dermatol* 2011; **36**: 292-297 [PMID: 21198791 DOI: 10.1111/j.1365-2230.2010.03972.x]
- 48 **Kolls JK**, Lindén A. Interleukin-17 family members and inflammation. *Immunity* 2004; **21**: 467-476 [PMID: 15485625 DOI: 10.1016/j.immuni.2004.08.018]
- 49 **Khan R**, Satyam A, Gupta S, Sharma VK, Sharma A. Circulatory levels of antioxidants and lipid peroxidation in Indian patients with generalized and localized vitiligo. *Arch Dermatol Res* 2009; **301**: 731-737 [PMID: 19488773 DOI: 10.1007/s00403-009-0964-4]
- 50 **Ines D**, Sonia B, Riadh BM, Amel el G, Slaheddine M, Hamida T, Hamadi A, Basma H. A comparative study of oxidant-antioxidant status in stable and active vitiligo patients. *Arch Dermatol Res* 2006; **298**: 147-152 [PMID: 16897080 DOI: 10.1007/s00403-006-0680-2]
- 51 **Latha B**, Babu M. The involvement of free radicals in burn injury: a review. *Burns* 2001; **27**: 309-317 [PMID: 11348738]
- 52 **Yildirim M**, Baysal V, Inaloz HS, Can M. The role of oxidants and antioxidants in generalized vitiligo at tissue level. *J Eur Acad Dermatol Venereol* 2004; **18**: 683-686 [PMID: 15482295]
- 53 **Dammak I**, Boudaya S, Ben Abdallah F, Turki H, Attia H, Hentati B. Antioxidant enzymes and lipid peroxidation at the tissue level in patients with stable and active vitiligo. *Int J Dermatol* 2009; **48**: 476-480 [PMID: 19416376]
- 54 **Sravani PV**, Babu NK, Gopal KV, Rao GR, Rao AR, Moorthy B, Rao TR. Determination of oxidative stress in vitiligo by measuring superoxide dismutase and catalase levels in vitiliginous and non-vitiliginous skin. *Indian J Dermatol Venereol Leprol* 2009; **75**: 268-271 [PMID: 19439879]
- 55 **Prota G**. The role of peroxidase in melanogenesis revisited. *Pigment Cell Res* 1992; **Suppl 2**: 25-31 [PMID: 1329073]
- 56 **Schallreuter KU**, Wood JM, Pittelkow MR, Gütlich I, Lemke KR, Rödl W, Swanson NN, Hitzemann K, Ziegler I. Regulation of melanin biosynthesis in the human epidermis by tetrahydrobiopterin. *Science* 1994; **263**: 1444-1446 [PMID: 8128228]
- 57 **Kowlessur D**, Citron BA, Kaufman S. Recombinant human phenylalanine hydroxylase: novel regulatory and structural properties. *Arch Biochem Biophys* 1996; **333**: 85-95 [PMID: 8806757]
- 58 **Hasse S**, Gibbons NC, Rokos H, Marles LK, Schallreuter KU. Perturbed 6-tetrahydrobiopterin recycling via decreased dihydropteridine reductase in vitiligo: more evidence for H2O2 stress. *J Invest Dermatol* 2004; **122**: 307-313 [PMID: 15009710]
- 59 **Iyengar B**. Modulation of melanocytic activity by acetylcholine. *Acta Anat (Basel)* 1989; **136**: 139-141 [PMID: 2510448]
- 60 **Schallreuter KU**, Elwary SM, Gibbons NC, Rokos H, Wood JM. Activation/deactivation of acetylcholinesterase by H2O2: more evidence for oxidative stress in vitiligo. *Biochem Biophys Res Commun* 2004; **315**: 502-508 [PMID: 14766237]
- 61 **Rakonczay Z**, Brimijoin S. Biochemistry and pathophysiology of the molecular forms of cholinesterases. *Subcell Biochem* 1988; **12**: 335-378 [PMID: 3043772 DOI: 10.1007/978-1-4899-1681-5\_10]
- 62 **Schallreuter KU**, Gibbons NC, Zothner C, Elwary SM, Rokos H, Wood JM. Butyrylcholinesterase is present in the human epidermis and is regulated by H2O2: more evidence for oxidative stress in vitiligo. *Biochem Biophys Res Commun* 2006; **349**: 931-938 [PMID: 16962996]
- 63 **Shalbaf M**, Gibbons NC, Wood JM, Maitland DJ, Rokos H, Elwary SM, Marles LK, Schallreuter KU. Presence of epidermal allantoin further supports oxidative stress in vitiligo. *Exp Dermatol* 2008; **17**: 761-770 [PMID: 18328088]
- 64 **Schallreuter KU**, Büttner G, Pittelkow MR, Wood JM, Swanson NN, Körner C. Cytotoxicity of 6-biopterin to human melanocytes. *Biochem Biophys Res Commun* 1994; **204**: 43-48 [PMID: 7945390]
- 65 **Schallreuter KU**, Wood JM, Ziegler I, Lemke KR, Pittelkow MR, Lindsey NJ, Gütlich M. Defective tetrahydrobiopterin and catecholamine biosynthesis in the depigmentation disorder vitiligo. *Biochim Biophys Acta* 1994; **1226**: 181-192 [PMID: 8204666]
- 66 **Orecchia G**, Frattini P, Cucchi ML, Santagostino G. Normal-range plasma catecholamines in patients with generalized and acrofacial vitiligo: preliminary report. *Dermatology* 1994; **189**: 350-353 [PMID: 7873818 DOI: 10.1159/000246877]
- 67 **Schallreuter KU**, Wood JM, Pittelkow MR, Büttner G, Swanson N, Korner C, Ehrke C. Increased monoamine oxidase A activity in the epidermis of patients with vitiligo. *Arch Dermatol Res* 1996; **288**: 14-18 [PMID: 8750929 DOI: 10.1007/BF02505037]
- 68 **Schallreuter KU**, Pittelkow MR, Wood JM. Free radical reduction by thioredoxin reductase at the surface of normal and vitiliginous human keratinocytes. *J Invest Dermatol* 1986; **87**: 728-732 [PMID: 2431070]
- 69 **Schallreuter KU**, Pittelkow MP. Defective calcium uptake in keratinocyte cell cultures from vitiliginous skin. *Arch Dermatol Res* 1988; **280**: 137-139 [PMID: 3377526 DOI: 10.1007/BF00456842]
- 70 **Schallreuter KU**, Wood JM. EF-hands calcium-binding regulates the thioredoxin reductase/thioredoxin electron transfer in human keratinocytes and melanocytes. In: Heizmann C, editor. Novel calcium-binding proteins. Berlin: Springer Verlag, 1991: 339-360
- 71 **Hann SK**, Chang JH, Lee HS, Kim SM. The classification of segmental vitiligo on the face. *Yonsei Med J* 2000; **41**: 209-212 [PMID: 10817021]
- 72 **Marks DB**, Marks AD, Smith CM. Oxygen metabolism and oxygen toxicity. In: Basic Medical Biochemistry a clinical Approach. Williams and Wilkins, Baltimore, Maryland, 1996: 327-340
- 73 **Bagherani N**, Yaghoobi R, Omidian M. Hypothesis: zinc can be effective in treatment of vitiligo. *Indian J Dermatol* 2011; **56**: 480-484 [PMID: 22121258]
- 74 **Yaghoobi R**, Omidian M, Bagherani N. Vitiligo: a review of the published work. *J Dermatol* 2011; **38**: 419-431 [PMID: 21667529]
- 75 **Gordon PR**, Mansur CP, Gilchrist BA. Regulation of human melanocyte growth, dendricity, and melanization by keratinocyte derived factors. *J Invest Dermatol* 1989; **92**: 565-572 [PMID: 2467948]
- 76 **Hassan MI**, Waheed A, Yadav S, Singh TP, Ahmad F. Zinc alpha 2-glycoprotein: a multidisciplinary protein. *Mol Cancer Res* 2008; **6**: 892-906 [PMID: 18567794 DOI: 10.1158/1541-7786.MCR-07-2195]
- 77 **Gauthier Y**, Cario Andre M, Taïeb A. A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? *Pigment Cell Res* 2003; **16**: 322-332 [PMID: 12859615]
- 78 **Gauthier Y**, Cario-Andre M, Lepreux S, Pain C, Taïeb A. Melanocyte detachment after skin friction in non lesional skin of patients with generalized vitiligo. *Br J Dermatol* 2003; **148**: 95-101 [PMID: 12534601]
- 79 **Whitton ME**, Ashcroft DM, González U. Therapeutic interventions for vitiligo. *J Am Acad Dermatol* 2008; **59**: 713-717 [PMID: 18793940]
- 80 **Bing C**, Bao Y, Jenkins J, Sanders P, Manieri M, Cinti S, Tisdale MJ, Trayhurn P. Zinc-alpha2-glycoprotein, a lipid mobilizing factor, is expressed in adipocytes and is up-regulated in mice with cancer cachexia. *Proc Natl Acad Sci USA* 2004; **101**: 2500-2505 [PMID: 14983038]
- 81 **Russell ST**, Tisdale MJ. The role of glucocorticoids in the induction of zinc-alpha2-glycoprotein expression in adipose tissue in cancer

- cachexia. *Br J Cancer* 2005; **92**: 876-881 [PMID: 15714206]
- 82 **Hale LP**. Zinc alpha-2-glycoprotein regulates melanin production by normal and malignant melanocytes. *J Invest Dermatol* 2002; **119**: 464-470 [PMID: 12190871]
- 83 **Boissy RE**, Spritz RA. Frontiers and controversies in the pathobiology of vitiligo: separating the wheat from the chaff. *Exp Dermatol* 2009; **18**: 583-585 [PMID: 19320739]
- 84 **Akbayir N**, Gökdemir G, Mansur T, Sökmen M, Gündüz S, Alkim C, Barutcuoglu B, Erdem L. Is there any relationship between hepatitis C virus and vitiligo? *J Clin Gastroenterol* 2004; **38**: 815-817 [PMID: 15365412]
- 85 **Akcan Y**, Kavak A, Serbas Y, Olut AI, Korkut E, Bicik Z, Kisacik B. The low seropositivity of hepatitis B virus in vitiligo patients. *J Eur Acad Dermatol Venereol* 2006; **20**: 110-111 [PMID: 16405627]
- 86 **Toker SC**, Sarycaoglu H, Karadogan SK, Mistik R, Baskan EB, Tunalı S. Is there any relation between vitiligo and cytomegalovirus? *J Eur Acad Dermatol Venereol* 2007; **21**: 141-142 [PMID: 17207202]
- 87 **Niamba P**, Traoré A, Taïeb A. [Vitiligo in a black patient associated with HIV infection and repigmentation under antiretroviral therapy]. *Ann Dermatol Venereol* 2007; **134**: 272-273 [PMID: 17389856]
- 88 **Boissy RE**, Liu YY, Medrano EE, Nordlund JJ. Structural aberration of the rough endoplasmic reticulum and melanosome compartmentalization in long-term cultures of melanocytes from vitiligo patients. *J Invest Dermatol* 1991; **97**: 395-404 [PMID: 1875040]
- 89 **Norris A**, Todd C, Graham A, Quinn AG, Thody AJ. The expression of the c-kit receptor by epidermal melanocytes may be reduced in vitiligo. *Br J Dermatol* 1996; **134**: 299-306 [PMID: 8746346]
- 90 **Wehrle-Haller B**. The role of Kit-ligand in melanocyte development and epidermal homeostasis. *Pigment Cell Res* 2003; **16**: 287-296 [PMID: 12753403]
- 91 **Lee AY**, Kim NH, Choi WI, Youm YH. Less keratinocyte-derived factors related to more keratinocyte apoptosis in depigmented than normally pigmented suction-blistered epidermis may cause passive melanocyte death in vitiligo. *J Invest Dermatol* 2005; **124**: 976-983 [PMID: 15854039]
- 92 **Tobin DJ**, Swanson NN, Pittelkow MR, Peters EM, Schallreuter KU. Melanocytes are not absent in lesional skin of long duration vitiligo. *J Pathol* 2000; **191**: 407-416 [PMID: 10918216]
- 93 **Kim YC**, Kim YJ, Kang HY, Sohn S, Lee ES. Histopathologic features in vitiligo. *Am J Dermatopathol* 2008; **30**: 112-116 [PMID: 18360112]
- 94 **Gottschalk GM**, Kidson SH. Molecular analysis of vitiligo lesions reveals sporadic melanocyte survival. *Int J Dermatol* 2007; **46**: 268-272 [PMID: 17343582]
- 95 **Falabella R**. Vitiligo and the melanocyte reservoir. *Indian J Dermatol* 2009; **54**: 313-318 [PMID: 20101329 DOI: 10.4103/0019-5154.57604]
- 96 **Davids LM**, du Toit E, Kidson SH, Todd G. A rare repigmentation pattern in a vitiligo patient: a clue to an epidermal stem-cell reservoir of melanocytes? *Clin Exp Dermatol* 2009; **34**: 246-248 [PMID: 18828846]
- 97 **Lee AY**, Youm YH, Kim NH, Yang H, Choi WI. Keratinocytes in the depigmented epidermis of vitiligo are more vulnerable to trauma (suction) than keratinocytes in the normally pigmented epidermis, resulting in their apoptosis. *Br J Dermatol* 2004; **151**: 995-1003 [PMID: 15541077]
- 98 **Bondanza S**, Maurelli R, Paterna P, Migliore E, Giacomo FD, Primavera G, Paionni E, Dellambra E, Guerra L. Keratinocyte cultures from involved skin in vitiligo patients show an impaired in vitro behaviour. *Pigment Cell Res* 2007; **20**: 288-300 [PMID: 17630962]
- 99 **van den Wijngaard R**, Wankowicz-Kalinska A, Le Poole C, Tigges B, Westerhof W, Das P. Local immune response in skin of generalized vitiligo patients. Destruction of melanocytes is associated with the prominent presence of CLA<sup>+</sup> T cells at the perilesional site. *Lab Invest* 2000; **80**: 1299-1309 [PMID: 10950121]
- 100 **Boissy RE**. Histology of vitiliginous skin. Hann S, Nordlund JJ, eds. Vitiligo, 1st ed. Oxford: Blackwell Science Ltd, 2008: 23-34
- 101 **Moretti S**, Fabbri P, Baroni G, Berti S, Bani D, Berti E, Nassini R, Lotti T, Massi D. Keratinocyte dysfunction in vitiligo epidermis: cytokine microenvironment and correlation to keratinocyte apoptosis. *Histol Histopathol* 2009; **24**: 849-857 [PMID: 19475531]
- 102 **Tschopp J**, Martinon F, Burns K. NALPs: a novel protein family involved in inflammation. *Nat Rev Mol Cell Biol* 2003; **4**: 95-104 [PMID: 12563287]
- 103 **Raj D**, Brash DE, Grossman D. Keratinocyte apoptosis in epidermal development and disease. *J Invest Dermatol* 2006; **126**: 243-257 [PMID: 16418733]
- 104 **Church LD**, Cook GP, McDermott MF. Primer: inflammasomes and interleukin 1beta in inflammatory disorders. *Nat Clin Pract Rheumatol* 2008; **4**: 34-42 [PMID: 18172447]
- 105 **Jin Y**, Bennett DC, Amadi-Myers A, Holland P, Riccardi SL, Gowan K, Fain PR, Spritz RA. Vitiligo-associated multiple autoimmune disease is not associated with genetic variation in AIRE. *Pigment Cell Res* 2007; **20**: 402-404 [PMID: 17850514]
- 106 **Jin Y**, Mailloux CM, Gowan K, Riccardi SL, LaBerge G, Bennett DC, Fain PR, Spritz RA. NALP1 in vitiligo-associated multiple autoimmune disease. *N Engl J Med* 2007; **356**: 1216-1225 [PMID: 17377159]
- 107 **Imokawa G**. Autocrine and paracrine regulation of melanocytes in human skin and in pigmentary disorders. *Pigment Cell Res* 2004; **17**: 96-110 [PMID: 15016298 DOI: 10.1111/j.1600-0749.2003.00126.x]
- 108 **Moretti S**, Spallanzani A, Amato L, Hautmann G, Gallerani I, Fabiani M, Fabbri P. New insights into the pathogenesis of vitiligo: imbalance of epidermal cytokines at sites of lesions. *Pigment Cell Res* 2002; **15**: 87-92 [PMID: 11936274]
- 109 **Gandarillas A**. Epidermal differentiation, apoptosis, and senescence: common pathways? *Exp Gerontol* 2000; **35**: 53-62 [PMID: 10705039]
- 110 **Cordisco S**, Maurelli R, Bondanza S, Stefanini M, Zambruno G, Guerra L, Dellambra E. Bmi-1 reduction plays a key role in physiological and premature aging of primary human keratinocytes. *J Invest Dermatol* 2010; **130**: 1048-1062 [PMID: 19907431]
- 111 **Chipuk JE**, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD, Schuler M, Green DR. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science* 2004; **303**: 1010-1014 [PMID: 14963330 DOI: 10.1126/science.1092734]
- 112 **Le Poole IC**, van den Wijngaard RM, Westerhof W, Das PK. Tenascin is overexpressed in vitiligo lesional skin and inhibits melanocyte adhesion. *Br J Dermatol* 1997; **137**: 171-178 [PMID: 9292062]
- 113 **Cario-André M**, Pain C, Gauthier Y, Taïeb A. The melanocytorrhagic hypothesis of vitiligo tested on pigmented, stressed, reconstructed epidermis. *Pigment Cell Res* 2007; **20**: 385-393 [PMID: 17850512]
- 114 **Halder RM**, Chappell JL. Vitiligo update. *Semin Cutan Med Surg* 2009; **28**: 86-92 [PMID: 19608058 DOI: 10.1016/j.sder.2009.04.008]
- 115 **Lebwohl MG**, Heymann WR, Berth-Jones J, Coulson I. Treatment of Skin Disease: Comprehensive Therapeutic Strategies. 2nd ed. Philadelphia, USA: Mosby Elsevier, 2006: 683-687
- 116 **Burns T**, Breathnach S, Cox N, Griffiths C. Rook's Textbook of Dermatology. 7th ed, Vol. II. Oxford: Blackwell Science, 2004: 52-57
- 117 **Wolff K**, Goldsmith LA, Katz SI, Gilchrist BA, Paller AS, Leffell DJ. Fitzpatrick's Dermatology in General Medicine. 7th ed, Vol. I. USA: Mac Graw Hill, 2007: 616-621
- 118 **Lotti T**, Gori A, Zanieri F, Colucci R, Moretti S. Vitiligo: new and emerging treatments. *Dermatol Ther* 2008; **21**: 110-117 [PMID: 18394085 DOI: 10.1111/j.1529-8019.2008.00178.x]
- 119 **Szczurko O**, Boon HS. A systematic review of natural health product treatment for vitiligo. *BMC Dermatol* 2008; **8**: 2 [PMID: 18498646 DOI: 10.1186/1471-5945-8-2]
- 120 **Bolognia JL**, Jorizzo JL, Rapini R. Dermatology. 2nd edn, Vol. I. USA: Mosby Elsevier, 2008: 913-920
- 121 **Forschner T**, Buchholtz S, Stockfleth E. Current state of vitiligo therapy--evidence-based analysis of the literature. *J Dtsch Dermatol Ges* 2007; **5**: 467-475 [PMID: 17537039]
- 122 **Grimes PE**. New insights and new therapies in vitiligo. *JAMA*

- 2005; **293**: 730-735 [PMID: 15701915]
- 123 **Ostovari N**, Passeron T, Zakaria W, Fontas E, Larouy JC, Blot JF, Lacour JP, Ortonne JP. Treatment of vitiligo by 308-nm excimer laser: an evaluation of variables affecting treatment response. *Lasers Surg Med* 2004; **35**: 152-156 [PMID: 15334620 DOI: 10.1002/lsm.20057]
- 124 **Pasricha JS**, Khera V. Effect of prolonged treatment with levamisole on vitiligo with limited and slow-spreading disease. *Int J Dermatol* 1994; **33**: 584-587 [PMID: 7960359]
- 125 **Tsuji T**, Hamada T. Topically administered fluorouracil in vitiligo. *Arch Dermatol* 1983; **119**: 722-727 [PMID: 6614958]
- 126 **van den Wijngaard R**, Wankowicz-Kalinska A, Pals S, Weening J, Das P. Autoimmune melanocyte destruction in vitiligo. *Lab Invest* 2001; **81**: 1061-1067 [PMID: 11502857]
- 127 **Michaëlsson G**, Juhlin L, Vahlquist A. Effects of oral zinc and vitamin A in acne. *Arch Dermatol* 1977; **113**: 31-36 [PMID: 137693]
- 128 **Hillström L**, Pettersson L, Hellbe L, Kjellin A, Leczinsky CG, Nordwall C. Comparison of oral treatment with zinc sulphate and placebo in acne vulgaris. *Br J Dermatol* 1977; **97**: 681-684 [PMID: 146511 DOI: 10.1111/j.1365-2133.1977.tb14277.x]
- 129 **Burrows NP**, Turnbull AJ, Punchard NA, Thompson RP, Jones RR. A trial of oral zinc supplementation in psoriasis. *Cutis* 1994; **54**: 117-118 [PMID: 7956335]
- 130 **Kim YJ**, Chung BS, Choi KC. Depigmentation therapy with Q-switched ruby laser after tanning in vitiligo universalis. *Dermatol Surg* 2001; **27**: 969-970 [PMID: 11737134]
- 131 **Koshevenko IuN**. [alpha-Tocopherol in the combined treatment of vitiligo]. *Vestn Dermatol Venerol* 1989; **10**: 70-72 [PMID: 2609752]
- 132 **Mandel AS**, Haberman HF, Pawlowski D, Goldstein E. Non PUVA nonsurgical therapies for vitiligo. *Clin Dermatol* 1997; **15**: 907-919 [PMID: 9404694 DOI: 10.1016/S0738-081X(97)00132-6]
- 133 **Bickers DR**, Athar M. Oxidative stress in the pathogenesis of skin disease. *J Invest Dermatol* 2006; **126**: 2565-2575 [PMID: 17108903 DOI: 10.1038/sj.jid.5700340]
- 134 **Fernandes G**. Dietary lipids and risk of autoimmune disease. *Clin Immunol Immunopathol* 1994; **72**: 193-197 [PMID: 8050192 DOI: 10.1006/clin.1994.1129]
- 135 **Logan AC**. Omega-3 fatty acids and major depression: a primer for the mental health professional. *Lipids Health Dis* 2004; **3**: 25 [PMID: 15535884 DOI: 10.1186/1476-511X-3-25]
- 136 **Joulain C**, Prigent AF, Némaz G, Lagarde M. Increased glutathione peroxidase activity in human blood mononuclear cells upon in vitro incubation with n-3 fatty acids. *Biochem Pharmacol* 1994; **47**: 1315-1323 [PMID: 8185640 DOI: 10.1016/0006-2952(94)90329-8]
- 137 **Namazi MR**. Prescribing cyclic antidepressants for vitiligo patients: which agents are superior, which are not? *Psychother Psychosom* 2003; **72**: 361-362 [PMID: 14526142]

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## Gall bladder carcinoma: Aggressive malignancy with protean loco-regional and distant spread

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poor prognosis and the 5 year survival rate is < 10%. Although etiology of the carcinoma of the gallbladder is still obscure, various factors have been implicated, cholelithiasis being the most frequent. The incidence of GBC worldwide is based on the gender, geography and ethnicity which suggest that both genetic and environmental factors can cause GBC. The major route of spread of gallbladder cancer (GC) is loco-regional rather than distant. It spreads by lymphatic, vascular, neural, intraperitoneal, and intraductal routes. Sonography is usually the most common imaging test to evaluate symptoms of biliary tract disease including suspected GC. With recent advances in imaging modalities like multi-detector computed tomography (CT) scanners, magnetic resonance imaging-positron emission tomography/CT diagnosis of gallbladder cancer has improved. Studies have also targeted molecular and genetic pathways. Treatment options have included extended and radical surgeries and adjuvant chemotherapy. This review article deals in detail with important aspects of carcinoma gallbladder and its manifestations and challenges. Role of various imaging modalities in characterization and accurate staging has been discussed. The loco-regional spread of this aggressive malignancy is dealt explicitly.

**Key words:** Gallbladder cancer; Loco-regional and distant spread; Cholelithiasis; Imaging; Adenocarcinoma

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**Core tip:** Gallbladder cancer is one of the most prevalent and lethal cancer of biliary tract with multi-factorial etiology. Cholelithiasis is the most common etiological factor. Adenocarcinoma is the most common histological type with loco-regional spread in majority of cases. Sonography is used widely as an initial screening tool and primary characterization of the tumor but it has a limited role in the diagnosis of early lesions. Thus, computed tomography and magnetic

### Abstract

The most common malignancy of biliary tract is gallbladder cancer (GBC) which is the third most common cancer in gastrointestinal tract. It is a lethal disease for most patients in spite of growing awareness and improved diagnostic techniques. GBC has a very



resonance imaging are used for complete morphologic characterization and staging of malignant gallbladder lesions and metastatic survey.

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## INTRODUCTION

Gallbladder cancer (GBC) is the most prevalent malignancy in biliary tract and third in gastrointestinal (GI) tract<sup>[1,2]</sup>. It was first described in detail in the seventies<sup>[3]</sup>. It is a lethal disease for most patients in spite of growing awareness and improved diagnostic techniques<sup>[4]</sup>. Although rare, it has high incidence in certain world populations<sup>[5]</sup>. GBC has poor prognosis and the survival rate in 5 years is less than 10%<sup>[5,6]</sup>. It affects predominantly women, four times more as compared to men<sup>[6,7]</sup>. GBC is asymptomatic in nature which makes it difficult in diagnosis and treatment. The symptoms of GBC are alike to other GI tract problems viz. abdominal pain, abdominal lump, anorexia, nausea, jaundice and vomiting<sup>[2]</sup>. GBC is a neoplasm which is widely known for its distinct ethnic, gender and geographical variations<sup>[8]</sup>. Precise etiology of GBC is still not known but various factors have been incriminated cholelithiasis<sup>[8]</sup>, external carcinogens<sup>[9]</sup>, free radicals, lipid peroxidation products, inflammatory bowel disease and secondary bile acids<sup>[10]</sup>. Congenital malformations of biliary tract (more common in Japan, China) are also observed as the risk factor for GBC. Chronic inflammatory conditions, heavy metals exposure, a high carbohydrate diet, obesity, alcohol abuse and smoking are also known as possible risk factor for GBC<sup>[11,12]</sup>. Adenocarcinoma is most prevalent histo-pathological type of GBC<sup>[8]</sup>.

## BACKGROUND AND EPIDEMIOLOGY

The incidence of GBC worldwide is based on the gender, geography and ethnicity which suggest that both genetic and environmental factors can cause GBC. Various epidemiological studies suggested that geographical and racial difference also affects the frequency of GBC. A high geographical variability in occurrence of GBC also correlated with the ubiquity of cholelithiasis<sup>[12]</sup>. Incidence rate of GBC is higher in South American Countries like Chile, Bolivia and Ecuador and some Asian countries like some areas of India, Pakistan, Japan, and South Korea<sup>[12]</sup>. Intermediate rate of incidence have been observed in European countries. Its rate is lower in United States but Native Americans observed to have a high incidence. Further,

urban areas have less risk compared to rural region. Incidence in Chile where mortality is 5.2% is highest in the world. There, it is the most prevalent cancer affecting women and the fourth important reason of cancer deaths<sup>[5]</sup>. Marked geographical differences seen in frequency of gallbladder carcinoma suggest a possible environmental cause besides race or ethnicity. The exact etiology of GBC has not been properly known till date. It is yet to be established. But several other factors like chronic cholecystitis, gallstones, choledochal cyst, female gender, age and exposure of carcinogens have been observed to be implicated in gallbladder carcinogenesis<sup>[13,14]</sup>. Randi *et al*<sup>[5]</sup> and other studies carried out where age adjusted incidence rates of GBC in various populations based on cancer registry data were considered concluded that maximum incidence rate was found in women in Delhi, India (21.5/100000), South Karachi, Pakistan (13.8/100000) and Quito, Ecuador (12.9/100000)<sup>[5,15,16]</sup>. Another study also described a very high incidence of cancer in Northern India (1.5/100000) and Native American Indian females (14.5/100000)<sup>[17]</sup>. Therefore, there is an urgent need of early detection of GBC<sup>[18]</sup>. Gastric cancer was the main cause of death in Japan since 1999. It was reported to be the sixth leading cause of cancer related death in Japan in 2007<sup>[19]</sup>. The incidence of GBC rises with age and reaches the peak during the seventh and eighth decades of life<sup>[6]</sup>. Age-standardized incidence rates (ASIR) has been found to be high above age of 45. Delhi has been listed as having the highest ASIR with 22.08 males and 35.67 females per 10<sup>5</sup> persons after the age of 65 years. Studies revealed a very distinct age-related pattern among both genders<sup>[20]</sup>. Female-to-male incidence ratio was generally around 3, but ranged from 1 in Far East Asia to over 5 in Spain and Columbia<sup>[5]</sup>. The incidence was observed to be higher from the Gangetic belt, but, due to lack of cancer registries in these areas, the precise incidence cannot be known accurately. The estimated occurrence of GBC in Varanasi is around 4.4% of all types cancers and around 16% of all GI cancers<sup>[2]</sup>.

## PATHOLOGY

Approximately 80% GBCs follow progression from dysplastic mucosa to carcinoma in situ and invasive carcinoma<sup>[21]</sup>. Morphological and molecular changes also suggest the same<sup>[22]</sup>. Approximately 60%, 30% and 10% tumors originate in the fundus, body and neck of the GB respectively<sup>[23]</sup>. Among all the GBC, adenocarcinoma constitute about 85% in comparison to others like epidermoid carcinomas (6.5%) and adenocanthomas (4.5%). Besides these, other histologic types of GBC are small (oat) cell carcinomas<sup>[24]</sup>, carcinoid tumors and anaplastic carcinomas<sup>[8]</sup>. Glandular, medullary, scirrhous, papillary, and colloid type are the parts of adenocarcinoma with incidence rate of 35.3%, 23.2%, 15.7% 14.5% and 11.3%

respectively<sup>[25]</sup>. Infiltrative, papillary and combined papillary-infiltrative forms are the different forms of GC<sup>[26]</sup>. Thickening and induration of GB wall occurred in infiltrating tumors which invades the subserosal plane and gallbladder wall into liver and neighboring structures. Papillary carcinoma shows a polypoid cauliflower like appearance which fills the GB lumen with very low wall invasion as it has the best prognosis. Recent researches also divide GC into metaplastic and non-metaplastic types<sup>[27]</sup>. Pseudopyloric and intestinal are two types of metaplastic variety. They are associated with chronic inflammation and cholelithiasis. Intestinal variety is associated with increasing age<sup>[28]</sup>.

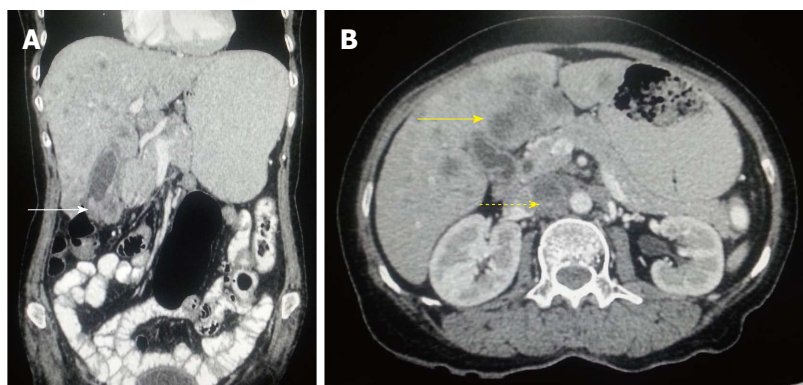
## MOLECULAR AND GENETICS RELATED TO GBC

Different epidemiological studies and diversity in incidence of GBC studies also suggest an association of gene in its etiopathogenesis. Few facts are available about genetic changes in GBC. *K-ras* gene mutations have been found in 39%-59% of GBC, whereas *p53* mutations have been reported in 35%-92% of patients having GBC<sup>[6,29]</sup>. Shukla *et al.*<sup>[30]</sup> reported that telomerase activity was significantly raised in gall bladder cancer tissue. Telomerase activity was mainly concentrated in poorly-differentiated adenocarcinomas (83.33%) and increased expression was present in advanced stages. The presence of telomerase may serve as a molecular marker for the diagnosis of gall bladder carcinoma and may have prognostic and therapeutic implications in the treatment of patients<sup>[30]</sup>. It has been reported in various studies that genetic alterations in *k-ras*, *p53* and *p16* take a significant part in gallbladder carcinogenesis<sup>[31-36]</sup>. Inactivation of tumor suppressor genes involves the different genetic mutational events which lead to the one allele and allelic loss of the other allele. This is the main and complex mechanism associated with it. This allelic loss is known as loss of heterozygosity (LOH) which can be detected by using microsatellite markers. Recurrent LOH has also been detected in GC at different chromosomal locations like 1p, 3p, 5q, 8p, 9q, 13q and 17p<sup>[37]</sup>. Other reports also suggested in their study that many chromosomal regions have important tumor suppressor genes which are also detected in this neoplasm. 3p (20% to 52%); 5q21 (*APC/MCC* gene, 6% to 66 %); 8p22-24 (22% to 44%); 13q14 (*RB* gene, 20% to 30%); and 18q (*DCC* gene, 18% to 31%) are the some gene locations other than the *TP53* and *CDKN* gene<sup>[37]</sup>. In all the cholecystitis and adenoma patients *Retinoblastoma* (*Rb*) gene have been detected; however, *Rb* gene is deleted in 18%-67% of carcinoma of the gallbladder patients<sup>[38,39]</sup>. The precise molecular abnormality which causes neoplastic transformation in the gallbladder epithelium still unclear. An accurate pathway related to molecular changes which further result in neoplastic

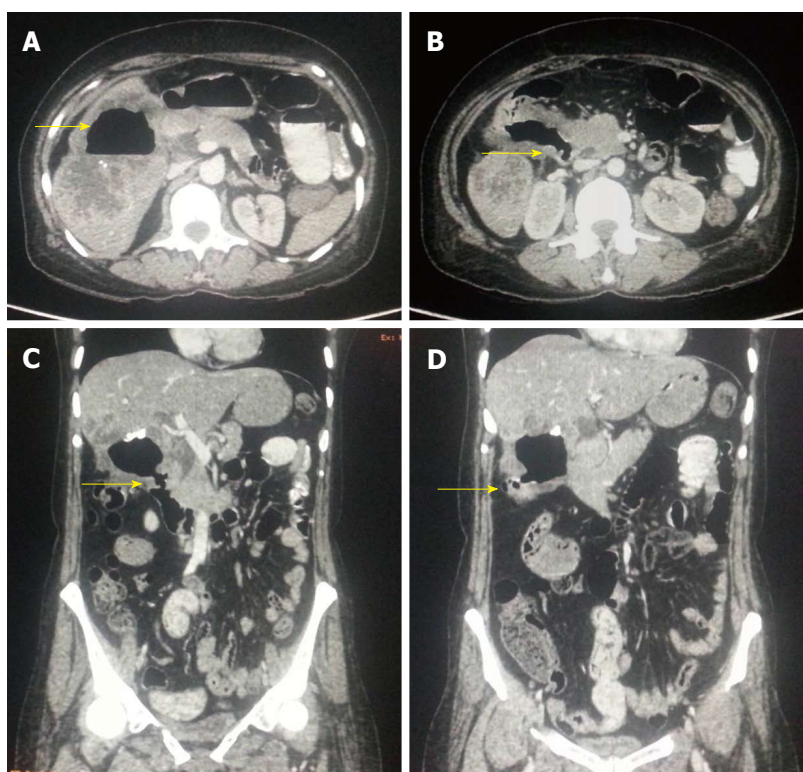
transformation in gallbladder epithelium has not fully understood. This more understanding of molecular changes events will give better tools to detect GBC in early stage.

## ROUTES OF SPREAD: LOCO-REGIONAL AND DISTANT

Vascular, lymphatic, intraperitoneal, neural and intraductal routes are the leading routes of spread. Intraductal spread in GBC has a better prognosis<sup>[40]</sup>. Invasion of liver and lymph nodes has been reported in 69% and 45% of patients respectively in 984 patients from nine series<sup>[8]</sup>. Loco regional spread in GC is more common than distant metastasis. Metastases usually occur in liver (Figures 1A-B), lymph nodes, adjacent organs and peritoneum. Lymph nodes are usually found in about 60% of cases while metastasis in liver is about 76%-86% cases. Intraperitoneal spread is common with ascites, omental nodules and peritoneal implants<sup>[41-43]</sup>. 76.8%, 71% and 24.6% cases had liver, lymph node involvement and peritoneal deposits respectively in a study consists of 69 patients which undergo exploratory laparotomy<sup>[1]</sup>. Lymphatic drainage from the gallbladder occurs in a predictive fashion and correlates with the pattern of lymph node metastasis seen in GBC. Initially, cystic duct and pericholedochal nodes are involved, followed by more distant metastasis to posterior nodes to the head of the pancreas and then to intraaortocaval lymph nodes. This primary route is called cholecysto-retropancreatic pathway. Secondary route of lymphatic drainage includes the retroportal and right celiac lymph nodes through the gastrohepatic ligament, called cholecysto-celiac pathway. The third one is called cholecysto-mesenteric route consisting of a pathway from the posterior of gallbladder to the aortocaval lymph nodes *via* pancreas<sup>[44]</sup>. It is common for GBC spread directly into the liver and porta hepatis which lead to narrowing or obstruction of the common hepatic or right hepatic duct. Surgical specimens from 48 patients who had undergone radical/extended cholecystectomies were examined in 5-mm stepwise tissue sections in one large surgical series<sup>[45]</sup>. Zero to three score was given to nodal spread (0: no metastases; 1: cystic, paradachal, hilar; 2: peripancreatic head, portal, hepatic artery; 3: celiac, periduodenal, perimesenteric). Out of 48 patients, 8 (17%), 7 (14.5%) and 9 (18.8%) were found to have group 1 and 3 nodes respectively. Out of 16 patients with group 2 or 3 nodes, only 3 were considered for curative resection. Such studies recommend a possible niche for regional radiation therapy where more distant nodal groups are commonly comprised for pancreatic cancer and cholangiocarcinoma (CC). Some studies also described that GBC have a greater risk for synchronous distant metastatic spread than in CC as GBC usually have a regional pattern of spread.



**Figure 1 Carcinoma gallbladder: Nodal and hepatic metastasis.** A: Coronal contrast-enhanced computed tomography (CECT) abdominal section shows relatively defined heterogenous mass involving fundus of gall bladder (arrow) with loss of fat plane with adjacent hepatic segment; B: Axial CECT abdominal section shows multiple hepatic metastasis (arrow) along with interaortocaval lymph nodal metastasis (dashed arrow).

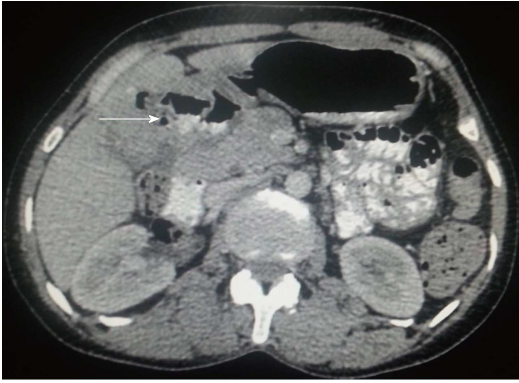


**Figure 2 Aggressive gallbladder cancer and entero-biliary fistula.** A: Axial contrast-enhanced computed tomography (CECT) abdominal section shows predominantly hypodense mass lesion replacing gall bladder fossa with presence of air (arrow) raising suspicion of fistula formation. Adjacent hepatic segments are also infiltrated by the mass; B and C: Axial and Coronal CECT abdominal sections clearly reveal the fistulous communication with D2 segment of duodenum (arrow); D: Coronal CECT abdominal section also show coexisting cholecysto-colonic fistula with hepatic flexure of colon (arrow).

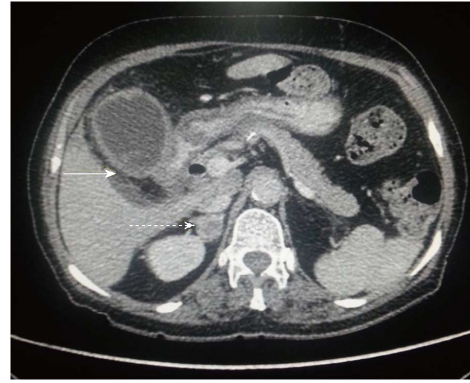
Ninety-seven patients of GBC and 76 patients of hilar CC were included in a study who were subjected to potentially curative resection and analyzed for patterns of initial disease recurrence<sup>[46]</sup>. GBC patients had a significantly lesser time to recurrence (11.5 mo for GBC, vs 20.3 mo;  $P = 0.07$ ) than hilar CC patients. In addition, GBC patients were far more likely to have a distant site involved at the initial period of recurrence (85% for GBC, vs 41%;  $P = 0.01$ ). They summarized in their study that patients of GBC were having less chances to benefit from locoregional therapy given the high rate of synchronous distant disease. Such conflicting results have made difficult to recommend chemo radiation. Spontaneous biliary-enteric fistulas are developed by gallstones (90%), peptic ulcer disease (6%) and malignancy or trauma (4%). Cholecysto-duodenal type (61% to 77%) is the most common communication, followed by cholecysto-colonic (14% to 17%) and cholecysto-gastric (6%)<sup>[47]</sup>

(Figures 2-5). Contrast cholangiographic studies were used in the diagnosis of gallbladder and biliary tract diseases before the emergence of USG and computed tomography (CT). Involvement of hepatic flexure and mesocolon has been observed in 33.3% of cases which was demonstrated by eccentric or circumferential wall thickening. A gallbladder mass lesion closely abutting hepatic flexure with no obvious eccentric wall thickening was observed in 2.3% cases<sup>[48]</sup>. According to Arminski<sup>[49]</sup> metastases occur to every organ including liver, lymph nodes, adrenal, kidney, spleen, brain, breast, thyroid, heart and uterus, those to the skeletal system are least frequent. Adrenal metastases (Figures 6 and 7) and venous occlusion due to tumor thrombus are unusual in newly diagnosed gallbladder carcinoma patients. Incidence of vascular metastasis is rare, but can occur. Portal vein invasion or tumor thrombus can be seen in aggressive or late cases. Vascular invasion leads to the localized involvement of





**Figure 3** Axial contrast-enhanced computed tomography abdominal section shows ill-defined heterogenous mass replacing gall bladder fossa with loss of fat plane with adjacent hepatic segments. Cholecystoduodenal and cholecysto-gastric fistulas are seen with D1 segment of duodenum and antropyloric region of stomach (arrow).



**Figure 6** Axial contrast-enhanced computed tomography abdominal section shows diffuse irregular nodular enhancing wall thickening of gall bladder (arrow) along with circumferential wall thickening of distal stomach and proximal duodenum. Associated right adrenal metastasis is also seen (dashed arrow).



**Figure 4** Axial contrast-enhanced computed tomography abdominal section shows ill-defined mass replacing gall bladder fossa with direct infiltration of hepatic segment V. Cholecystoduodenal fistula (arrow) is noted along with retroperitoneal lymph nodal metastasis (dashed arrow).



**Figure 7** Axial contrast-enhanced computed tomography abdominal section shows ill-defined heterogenous mass replacing gall bladder fossa with direct infiltration of adjacent hepatic segments. Associated left adrenal lesion is also seen (dashed arrow) raising suspicion of adrenal metastasis.

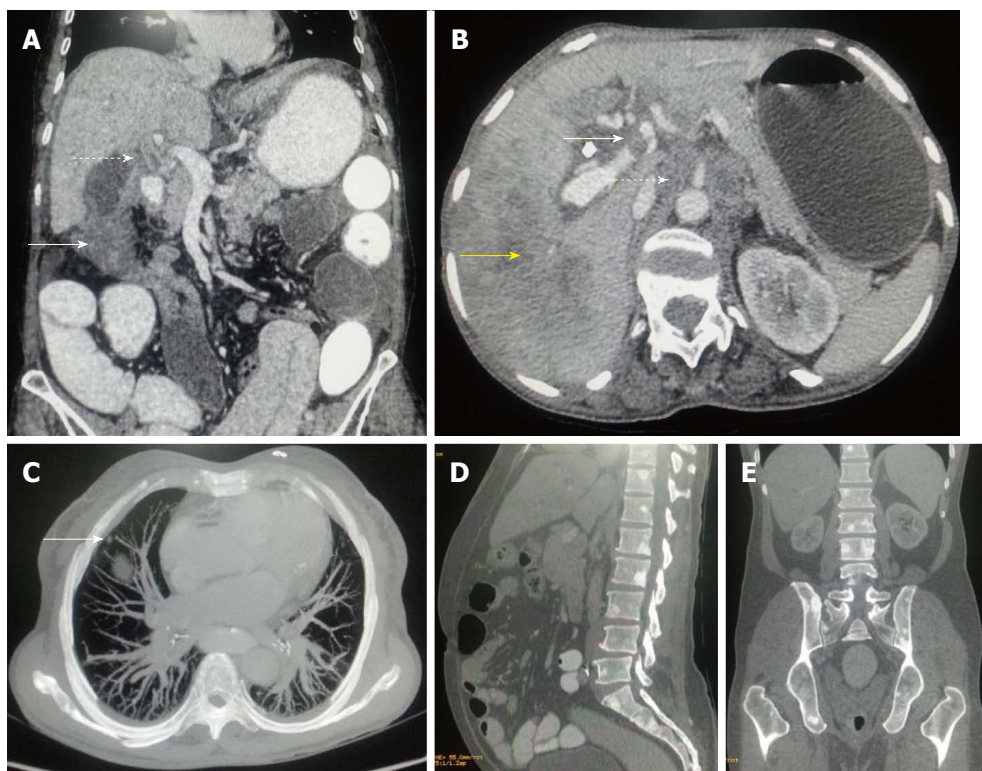


**Figure 5** Axial contrast-enhanced computed tomography abdominal section shows ill-defined heterogenous mass replacing gall bladder fossa with direct infiltration of hepatic segments. Cholecystoduodenal fistula (arrow) is noted along with multiple hepatic metastasis (dashed arrow).

the liver in the neighboring primary lesion preferably than disseminated multiple nodules<sup>[50]</sup>. Disseminated metastases appear in the late stage of the disease and

caused due to retroperitoneal veins invasion. Median survival rate in patients of carcinoma gallbladder with distant metastases is only 3 to 4 mo and these patients may not be offered any intervention<sup>[42]</sup>. Indian studies suggest that cases from this geographic belt are more aggressive<sup>[51]</sup>. Accuracy rate of preoperative staging by using multislice CT has an overall range from 83% to 86%<sup>[52,53]</sup>. GBC can have myriad of manifestations and spread<sup>[54]</sup>. The rarity of bony metastases from primary carcinoma has been documented by other authors<sup>[49,55-57]</sup>. In cases of skeletal metastases of the carcinoma of the gall bladder, 90% are purely osteolytic, 10% are mixed lytic and blastic type with purely osteoblastic lesions being unknown<sup>[58]</sup> (Figures 8). GC have non-specific laboratory finding. Most common laboratory finding are liver function abnormality. Serum alkaline phosphatase (ALP), direct bilirubin (conjugated bilirubin), and aspartate aminotransferase (AST) concentrations are usually deranged in more than 50% of cases. The patient is mildly hypoalbuminemic and hemoglobin level is





**Figure 8 Gallbladder carcinoma: regional and distant metastasis.** A: Axial contrast-enhanced computed tomography abdominal section shows heterogeneous mass lesion involving fundus of gall bladder fossa (arrow) with associated circumferential enhancing wall thickening of CBD (dashed arrow) indicating cholangitis or intraductal spread of malignancy; B: Axial CECT abdominal section shows hypodense filling defect in left branch of portal vein indicating thrombus formation (arrow). Hepatic infiltration is seen in segment VII (yellow arrow) with retroperitoneal lymph nodal metastasis (dashed arrow); C: MIP axial CT imaging shows soft tissue nodule in anterior segment of right lung indicating pulmonary metastasis (arrow); D and E: Sagittal and coronal CECT abdominal sections (bone window) show multiple osteoblastic skeletal metastatic lesions. CBD: Corticobasal degeneration; CECT: Contrast-enhanced computed tomography.

usually less than 11 g/dL in 10% of patients<sup>[59]</sup>.

## STAGING

Staging is the main part for the management and reporting of GBC. Gallbladder carcinoma is staged primarily at the time of surgery. Pathologic staging is decided at the time of surgery when the resection has been performed. Resection area (R) should be reported as it acts as the most important prognostic factor for GBC<sup>[60]</sup>. American Joint Committee on Cancer has given a TNM Staging System which usually determined by the depth of invasion, expansion of GC into adjacent structures, lymph node involvement and metastatic spread<sup>[61,62]</sup>. The primary stage of GC which decides the treatment is the "T" stage. Surgery is usually performed for T1/T2 (tumor confined to GB wall) if metastasis is absent. Tumors extending beyond the GB wall are considered T3 and T4. T3 tumors can be resected but with en bloc resection of adjacent organs while T4 tumors are unresectable<sup>[17]</sup>. But a simple cholecystectomy cannot be used to completely remove a T2 stage tumor as there is no serosa on the GB on the side from where it is attached to liver<sup>[60]</sup>. A minimum presence of three regional lymph nodes is required for accurate "N" staging. Hilar, celiac, periduodenal, peripancreatic and superior mesenteric

nodes as well as nodes along the pancreatic head are included in the regional lymph nodes. Lymph nodes outside the hepato-duodenal ligament are regarded as metastatic disease<sup>[17]</sup>. Metastasis to liver and peritoneum is common and occasionally to the lungs and pleura. Direct tumor invasion to the liver should not be regarded as distant metastasis<sup>[60]</sup>.

## IMAGING MODALITIES

### Abdominal ultrasound

The most prominent imaging modality to assess symptoms of biliary tract disease along with suspected GC is Ultrasound (US). It can differentiate between carcinoma and chronic cholecystitis with a sensitivity of 44% in early stages of disease<sup>[63]</sup>. Sensitivity differs for different types as reported by a retrospective study using US in early GC<sup>[64]</sup>. Ultrasound can easily detect invasion of liver parenchyma and loss of normal tissue interface. The sensitivity and accuracy of US in advanced GC are 85% and 80% respectively<sup>[65]</sup>. Bach *et al*<sup>[66]</sup> in their study reported the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in non-resectable carcinoma. The gallbladder wall is not visualized or poorly visualized as a thin echogenic line on ultrasound. An echogenic double-rim effect is produced when the gallbladder wall

becomes thickened from wall edema in inflammatory diseases<sup>[67]</sup>. Increase in gallbladder wall thickness can be seen in association with many conditions including chronic cholecystitis and neoplasia<sup>[68]</sup>. Sonography is often the first requested imaging technique in suspicion of gallbladder diseases due to cost effectiveness and easy availability<sup>[69-71]</sup>. CT and MRI are preferred over US for detection of early lesions and accurate staging and characterization<sup>[69,71,72]</sup>. US should be used along with other modalities as a complementary tool<sup>[73]</sup>. Hederström *et al.*<sup>[63]</sup> in their study concluded that US had poor sensitivity to differentiate GBC from chronic cholecystitis. There is significant chance of missing a malignancy. A cholecystectomy in such cases is useful. Another study reported that 6 were correctly diagnosed by surgery or autopsy out of 11 ultrasonographically proved GC. It concluded that sonography can suggest the diagnosis of gallbladder carcinoma, but inflammatory changes in the gallbladder may simulate or mask the signs of malignancy<sup>[74]</sup>. A study by Wibbenmeyer *et al.*<sup>[75]</sup> suggested that several sonographic findings were more common in patients with GBC in comparison to patients with benign gallbladder pathologies. Evaluation of these signs may be helpful in such conditions<sup>[75]</sup>. Doppler US has been found to be useful in detecting invasion of GC into the liver, the portal vein, and the bile ducts, but it has limitation in detection of lymph node and peritoneal metastases<sup>[66]</sup>. Contrast enhanced US may be helpful in improving the diagnosis of GC. The usefulness of contrast enhanced US for differential diagnosis of polypoid GB lesions has been described by Hattori *et al.*<sup>[76]</sup>. Classification of contrast enhanced patterns were described as linear, scattered, and diffuse or branched. It had a sensitivity of 100%, specificity of 76.9% and accuracy of 84.5% in case of diffuse or branched type pattern<sup>[76]</sup>. US guided fine needle aspiration cytology (FNAC) has been routinely for tissue diagnosis. It has an accuracy of 95% without major complications<sup>[77]</sup>. FNAC has been reported to have a sensitivity of 88%, specificity of 100%, PPV of 100% and NPV of 52%<sup>[78]</sup>.

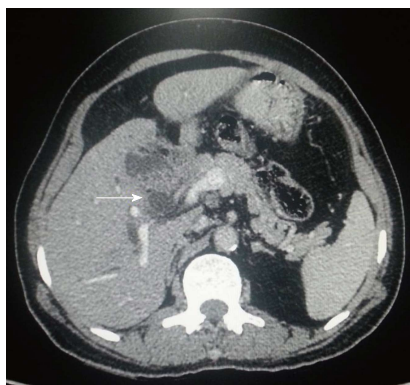
### Endoscopic ultrasonography

Endoscopic ultrasonography (EUS) has been widely used for peri-operative staging of GC. It looks as a hypoechoic mass with or without GB wall calcifications<sup>[79]</sup>. The overall accuracy rate of 91.9% has been reported in differentiating neoplastic from non-neoplastic masses<sup>[80]</sup>. Mitake *et al.*<sup>[81]</sup> also demonstrated the effectiveness of EUS in determination of the extent of tumor invasion and GC diagnosis. They reported an overall accuracy rate for tumor invasion depth to be 76.5% and differentiation between early and advanced stage tumors was possible in 79.5%. Furthermore, the usefulness of EUS has increased by the development of contrast agents. The depth of GC invasion was accurately assessed in 11 of 14 cases (78.6%) by conventional EUS, and in 13 of 14 cases (92.9%) by

contrast enhanced EUS in a comparative study. If we compare the conventional ultrasonography to endoscopic ultrasonography, the endoscopic ultrasonography has been observed to be more precise for staging<sup>[82,83]</sup>. A combination of diagnostic methods and biopsies may bring down the incidence of explorative laparotomies performed for GC<sup>[71]</sup>. Other aids like percutaneous liver biopsies, cholecystocentesis and culture of bile have been suggested in diagnosis of equivocal cases<sup>[84]</sup>. Use of EUS alone or in combination with EUS-guided FNA of gallbladder can improve the diagnosis of GC<sup>[85,86]</sup>. EUS-guided FNA is a safe and reliable modality for carcinoma gallbladder<sup>[79]</sup>.

### CT

CT is a common imaging modality for the identification of primary tumor and tumor staging. Hepatic parenchyma is the most commonly invaded site, followed by the bile duct and neighboring organs. CT scan can also accurately determine peritoneal and lymphatic metastasis<sup>[87]</sup>. Pericholedochal nodes and cystic nodes are commonly involved<sup>[88]</sup>. Kim *et al.*<sup>[89]</sup> using preoperative CT reported a precision of 71%. Helical CT was suggested to evaluate the spread and depth invasion of GC to assess resectability<sup>[90]</sup>. There are many advantages of helical CT over conventional CT<sup>[52]</sup>. In a retrospective study diagnostic precision for T staging was reported to be 83%-86% in comparison to conventional CT<sup>[52]</sup>. In another study overall accuracy of CT for staging GC was 71% and 79% for T1 and T2 tumors, 46% for T3 tumors, and 73% for T4 tumors. There was statistically significant difference between thickened wall and intraluminal mass type of tumors ( $P < 0.05$ ). The accuracy was (89%) for the intraluminal mass type, (83%) for massive type, while it was 54% in the thickened wall type<sup>[89]</sup>. Another prospective Japanese study using spiral CT reported affectability, specificity, PPV, NPP and the general exactness to be 88%, 87%, 88%, 87%, and 87%, respectively<sup>[91]</sup>. Although CT is not routinely used to explore patients with gallbladder disease symptoms, it is an important examination for suspected cases of gallbladder carcinoma. The most widely recognized CT finding in gallbladder carcinoma is a mass that fills the greater part of an irregular and distorted gallbladder<sup>[92]</sup> (Figure 9). Gallbladder carcinoma appears as a symmetric or asymmetric gallbladder wall thickening that may be hard to distinguish from the scarred gallbladder seen in chronic cholecystitis. Non-specific gallbladder wall thickening can be seen in acute and chronic cholecystitis, xanthogranulomatous cholecystitis, adenomyomatosis, diffuse hepatic or systemic disorders like hepatitis, portal hypertension, and congestive heart failure<sup>[46,93]</sup>. Yun *et al.*<sup>[87]</sup> used dual phase CT to assess thickness as well as enhancement pattern of gallbladder wall seen in gallbladder melanoma as well as chronic cholecystitis in arterial and venous phase. They reported a difference in enhancement patterns of malignancy as compared to chronic cholecystitis using



**Figure 9** Axial contrast-enhanced computed tomography abdominal section shows heterogenous mass involving gall bladder fossa and leading to obstruction of biliary system due to intraductal spread (arrow).

dual phase CT. Kim *et al.*<sup>[89]</sup> assessed the enhancement pattern of abnormal GB wall thickening using MDCT to differentiate between carcinoma and inflammatory diseases. They concluded that there is a distinct pattern of enhancement of inner wall compared to non-enhancing surface covering. Different signs of gallbladder carcinoma can be found due to biliary obstruction and liver involvement<sup>[53]</sup>. CT was found to be 85% precise in evaluation loco-regional spread of gallbladder malignancy<sup>[94]</sup>. It can be useful in guiding aspiration/biopsy from gallbladder in few cases<sup>[95]</sup>. It is prudent to correlate CT scan findings with clinical and laboratory findings in elderly individuals, particularly women presenting with acute cholecystitis and abnormal liver function. Imaging findings suspicious of carcinoma are diffuse irregular gallbladder wall thickening, intraluminal mass along with enlarged local lymph nodes<sup>[96]</sup>. Lee *et al.*<sup>[97]</sup> compared the efficacy of ultrasound (US) and CT in cases of intraluminal and infiltrating gallbladder carcinoma with and without gallstones. They found CT to be superior than US. Some authors suggest that combination of CT with ultrasound increases the diagnostic accuracy of gallbladder carcinoma associated with cholecystitis<sup>[98]</sup>.

### **Magnetic resonance imaging**

Initially due to disadvantage of poor spatial and contrast resolution, magnetic resonance imaging (MRI) was not widely used to evaluate GB disease<sup>[18]</sup>. Nonetheless, with recent advances and introduction of dynamic techniques after the administration of paramagnetic contrast, MR cholangiopancreatography (MRCP) and MR angiography (MRA), have been utilized for tumor staging<sup>[70]</sup>. Primary GC appears hypodense on T1 weighted images and hyperdense on T2 images with sensitivity of 67%-100% and specificity of 89%-100%<sup>[95]</sup>. The detection rate of lymph node metastasis was only (57%). The sensitivity and specificity for vascular invasion can be increased by combining MR cholangiography with three-dimensional MR angiography<sup>[99,100]</sup>.

### **Positron emission tomography**

18F-2fluoro-2-deoxy- D-glucose (FDG) uptake by tumor cells gives positron emission tomography (PET) imaging the combined benefit of utilizing metabolic activity and imaging features together. The main drawback of FDG-PET is that it is not yet generally accessible for routine clinical use and the low pervasiveness of GC. Till date, there is little data on the feasible contribution of these techniques in the useful imaging diagnosis of GC. In a recent prospective cohort study in patients presenting with radiologically suspicious gallbladder lesions<sup>[101]</sup> a staging diagnostic pre-surgical FDG-PET study was performed. Total diagnostic precision was 83.33% for the finding of the primary lesion, 88.89% for the assessment of involvement of lymph node and 85.1% for the assessment of metastatic spread. Karim *et al.*<sup>[102]</sup> examined the efficiency of different imaging techniques employed in GBC diagnosis.

### **Preoperative evaluation and staging**

MDCT is widely available nowadays and has a reported precision of up to 84% in determining the T stage of primary gallbladder carcinoma<sup>[103]</sup> and 85% in foreseeing resectability due to its capacity to depict hepatic and vascular invasion, lymph nodal and distant metastases<sup>[104]</sup>. MDCT is commonly performed as unenhanced and contrast-enhanced dual phase, from which multiplanar and 3D volume-rendered reconstruction images are generated to provide complete anatomic information. Additional coronal oblique images may be acquired for surgical management<sup>[104]</sup>. Kim *et al.*<sup>[89]</sup> claimed that the all-in-one standard protocol of using MR cholangiopancreatography and contrast enhanced MR angiography may yield a sensitivity of up to 100% with regard to bile duct and vascular invasion. However it is only 67% with regard to hepatic involvement and 56% with regard to lymph node metastases. PET/CT may have a promising role in the diagnosis associated with unsuspected metastases, which might change staging and treatment<sup>[105,106]</sup>. To date, potential research which specifically assess CT, MRI, and PET/CT in their capabilities to detect and stage gallbladder carcinoma are yet to be executed. The particular spread of gallbladder carcinoma to the liver parenchyma in addition to surrounding internal organs is possibly due to lack of muscularis mucosa in addition to submucosa inside the gallbladder wall structure and primary venous drainage with the liver parenchyma on the hepatic abnormal veins. As per the sixth release of American Joint Committee on Cancer staging manual for gallbladder carcinoma<sup>[107]</sup> primary gallbladder carcinoma can be delegated T1, kept to the lamina propria or the muscle layer of the gallbladder (T1a and T1b, separately); T2, stretching out to the serosa; T3, puncturing the serosa or directly invading the liver or one other contiguous structure (stomach, duodenum,



colon, pancreas, omentum, extrahepatic bile channels); or T4, invading the primary portal vein, the hepatic artery, or numerous extrahepatic organs. Lymphatic spread is present in more than half of patients at initial finding and first reaches cystic, pericholedochal, hilar, periduodenal, peripancreatic, and predominant mesenteric nodes, which are viewed as regional or N1 nodes. Portacaval, inter-aortocaval, and more distant nodes are viewed as distant or M1 disease. Gallbladder carcinoma can spread through intraductal route along the cystic duct, hematogenous route and neural pathways, and intraperitoneal "drop" metastases<sup>[70]</sup>. T1 or T2 involvement without nodal metastasis are termed as stage I A or I B respectively. T3 disease without nodal spread are stage II A. T1, T2, or T3 disease with N1 lymph node involvement is characterized as stage II B. A T4 disease without distant metastasis is considered as stage III. Any patient with distant disease comes in to the category of stage IV.

### Cytology

A carcinoma at an early stage can be ignored, and the diagnosis can only be made after microscopic examination of paraffin-embedded tissue. Imprint cytology of the gallbladder mucosa is a simple, quick, and excellent method for the detection of GBC<sup>[108]</sup>. Ultrasound-guided fine-needle aspiration cytology is likewise a safe diagnostic method for GBC<sup>[109]</sup>. Endoscopic retrograde cholangio-pancreaticography of biliary tree and GBC can also be considered for assessment of clinically suspicious carcinoma<sup>[110]</sup>.

### Tumor markers

Nowadays, tumor markers have a significant role in the detection and assessment of GBC. Investigation of CA242, CA15-3, CA19-9, and CA125 are genuinely proficient markers for segregating patients of carcinoma of the gallbladder from cholelithiasis. CA242 and CA125 when utilized together accomplished best sensitivity and specificity. Serum markers appear to be less effective when utilized independently, however it can be a useful complementary tool in combination<sup>[111]</sup>. No biochemical markers are useful in early detection of GC. Cholestasis and hyperbilirubinemia indicate late stage disease. Carcinoembryonic antigen (CEA) more than 4 ng/mL has a sensitivity of 50% and specificity of 93%<sup>[112]</sup>. Presence of CA19-9 suggest poor prognosis. A value more than 20 IU/mL has a specificity of 79%. CA19-9 is frequently increased in the presence of biliary obstruction therefore it is less specific in patients of jaundice<sup>[112]</sup>. Increased levels of serum alpha fetoprotein (AFP) have been seen in few patients but have little significance<sup>[113]</sup>.

### Electrophoretic pattern of proteins

Electrophoretic examination of serum protein has demonstrated protein bands in patients of carcinoma of the gallbladder in comparison to electrophoretic

pattern in cholelithiasis<sup>[114]</sup>.

### Gallbladder membrane lipids

Fourier enhance infrared (FTIR) spectroscopy is usually very sensitive for the molecular composition of tissues, and has the potential to distinguish premalignant tissues. Lipids have been elevated inside the plasma tissue layer of GBC. This proportion associated with high intensity might be a marker to help in identification of cancer through FTIR<sup>[115]</sup>.

## TREATMENT OPTIONS

The only likely curative treatments intended for gallbladder carcinoma is usually surgical resection. Unfortunately, most patients with GBC have unresectable disease. Only 10%-30% of patients can be considered for surgery on presentation<sup>[29]</sup>. The surgical alternatives for the treatment of GBC have evolved over the years. The methods range from a simple cholecystectomy to a radical or extended cholecystectomy, which incorporates the gallbladder in addition to 2 cm of liver tissue from the gallbladder bed. The radical cholecystectomy has been further modified to incorporate more significant liver resections, like segmentectomies (4b/5), right hepatectomies and trisectionectomy. Extended procedures additionally incorporate regional lymphadenectomy of the porta hepatis and periduodenal and pancreatic stations. Few surgeons incorporate a resection of the bile duct to clear the lymphatics in the porta hepatis. A few specialists now incorporate periaortic lymph node dissection for staging purposes and if the tumor is distal or includes the head of the pancreas, a pancreato-duodenectomy is added to accomplish R0 resection status<sup>[116,117]</sup>. Incidental GBC is found during cholecystectomy in 1%-2% of the cases. GBC should be suspected in the event of a tough gallbladder dissection or if there is presence of regional lymphadenopathy. A simple cholecystectomy is definitely an adequate treatment for Tis and T1 stage. Five-year survival for patients with T1 tumors is more than 85% with simple cholecystectomy<sup>[117,118]</sup>. For the T1 stage patients, the estimation of radical resection depends upon whether it is a T1a tumor or T1b tumor. In a study, the authors compared treatment of simple cholecystectomy and radical resection of T1a patients; however they didn't find any variation in survival or recurrence in the two groups<sup>[119]</sup>. Additionally, no positive lymph nodes were found in the sampled 147 lymph nodes in 12 patients who underwent radical resection. Simple cholecystectomy is needed for T1a GBC. For T1b (muscle invasion), there is proof that a more aggressive surgical methodology is needed. T1b tumors had lymph node metastases in 15% of cases, though only 2.5% of T1a tumors are accounted to have lymph node association<sup>[117]</sup>. Extrahepatic biliary resection is advocated for the management of T3 and T4 tumors<sup>[120]</sup>. The authors propose that the limit for extrahepatic



biliary resection ought to be decreased in patients with penetration of GBC through the subserosa<sup>[120]</sup>. Adjuvant combination chemotherapy and molecular targeted therapy are emerging as powerful therapeutics choices in those with advanced GBC. These days, adjuvant combination chemotherapy and molecular targeted therapy are regularly utilized as viable treatment for advanced GBC<sup>[121,122]</sup>. Adjuvant radiation treatment is utilized in locally advanced GBC or gallbladder disease with regional disease and has better survival rate<sup>[123,124]</sup>. It has been found that chemotherapy did not give effective treatment to unresectable GBC. Different regimens are already studied which include mixtures of 5-FU, leucovorin, mitomycin, adriamycin, in addition to nitrosoureas. A study observed a 64% reaction rate to gemcitabine with or without cisplatin to patients with stage 4 GBCs during phase II trial. Average time to development was 28 wk and the average general survival was 42 wk<sup>[125]</sup>. In other phase II trial utilizing a combination of gemcitabine and carboplatin in 20 patients with unresectable GBC, the reaction rate was 37%, average time to development was 34 wk, and the 1-year survival rate was 43%. The study concluded that chemotherapy with mixture of gemcitabine and carboplatin is viable in the treatment of advanced gallbladder carcinoma<sup>[126]</sup>. Palliative treatment may be done if GBC is found to be unresectable at the time of surgical investigation. The particular rate of biliary obstruction within affected individuals by GBC is higher than 60%<sup>[117]</sup>. A study concluded that combination of gemcitabine and oxaliplatin works well in inoperable GBC with overall response rate of more than 20%. It depicted that it may even incite complete pathological reaction. One year survival was found in more than 20% patients<sup>[127]</sup>. Other phase III study predicts that survival rate has been found higher in those patients who receive combination of gemcitabine and cisplatin in comparison to gemcitabine alone. Different studies are in process of clinical trials for molecular targeted agents which restrain angiogenesis and EGFR pathways<sup>[128]</sup>. New effective treatment and drugs are an urgent need for GBC. Recently a new study has been done in which the authors look at the effects of triptolide on GBC cells to identify its anticancer effects. They concluded in their study that triptolide induce apoptosis in gallbladder cells and thus can be used as a potential drug for treatment of GBC<sup>[129]</sup>.

## CONCLUSION

The clinical and radiologic diagnosis of gallbladder carcinoma at an early stage is challenging. It is crucial for radiologists to examine the gallbladder in its entirety, especially in patients who are at a greater risk of developing GBC, for important morphologic abnormalities that may suggest carcinoma. Identification of characteristic imaging appearances of primary GBC and comprehension of its pathways of spread and staging criteria will help in

formulating appropriate treatment regimens.

## REFERENCES

- 1 **Pandey M**, Pathak AK, Gautam A, Aryya NC, Shukla VK. Carcinoma of the gallbladder: a retrospective review of 99 cases. *Dig Dis Sci* 2001; **46**: 1145-1151 [PMID: 11414285]
- 2 **Shukla VK**, Khandelwal C, Roy SK, Vaidya MP. Primary carcinoma of the gall bladder: a review of a 16-year period at the University Hospital. *J Surg Oncol* 1985; **28**: 32-35 [PMID: 3968886 DOI: 10.1002/jso.2930280109]
- 3 **Solan MJ**, Jackson BT. Carcinoma of the gall-bladder. A clinical appraisal and review of 57 cases. *Br J Surg* 1971; **58**: 593-597 [PMID: 5558173 DOI: 10.1002/bjs.1800580814]
- 4 **Paimela H**, Karppinen A, Höckerstedt K, Perhoniemi V, Vaittinen E, Kivilaakso E. Poor prognosis of gallbladder cancer persists regardless of improved diagnostic methods. Incidence and results of surgery during 20 years in Helsinki. *Ann Chir Gynaecol* 1997; **86**: 13-17 [PMID: 9181213]
- 5 **Randi G**, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. *Int J Cancer* 2006; **118**: 1591-1602 [PMID: 16397865 DOI: 10.1002/ijc.21683]
- 6 **Lazcano-Ponce EC**, Miquel JF, Muñoz N, Herrero R, Ferrecio C, Wistuba II, Alonso de Ruiz P, Aristi Urista G, Nervi F. Epidemiology and molecular pathology of gallbladder cancer. *CA Cancer J Clin* 2001; **51**: 349-364 [PMID: 11760569 DOI: 10.3322/canjclin.51.6.349]
- 7 **Puhalla H**, Bareck E, Scheithauer W, Ploner M, Stiglbauer W, Depisch D. [Therapy of gallbladder carcinoma. Experience of a central hospital]. *Chirurg* 2002; **73**: 50-56 [PMID: 11974462 DOI: 10.1007/s104-002-8029-7]
- 8 **Piehl JM**, Crichtlow RW. Primary carcinoma of the gallbladder. *Surg Gynecol Obstet* 1978; **147**: 929-942 [PMID: 362580]
- 9 **Gradsar IA**, Kelly TR. Primary carcinoma of the gallbladder. *Arch Surg* 1970; **100**: 232-235 [PMID: 5414508 DOI: 10.1001/archsurg.1970.01340210008003]
- 10 **Shukla VK**, Shukla PK, Pandey M, Rao BR, Roy SK. Lipid peroxidation product in bile from patients with carcinoma of the gallbladder: a preliminary study. *J Surg Oncol* 1994; **56**: 258-262 [PMID: 8057656 DOI: 10.1002/jso.2930560415]
- 11 **Serra I**, Calvo A, Báez S, Yamamoto M, Endoh K, Aranda W. Risk factors for gallbladder cancer. An international collaborative case-control study. *Cancer* 1996; **78**: 1515-1517 [PMID: 8839560]
- 12 **Diehl AK**. Epidemiology of gallbladder cancer: a synthesis of recent data. *J Natl Cancer Inst* 1980; **65**: 1209-1214 [PMID: 6933267]
- 13 **Chao TC**, Greager JA. Primary carcinoma of the gallbladder. *J Surg Oncol* 1991; **46**: 215-221 [PMID: 2008087 DOI: 10.1002/jso.2930460402]
- 14 **Carriaga MT**, Henson DE. Liver, gallbladder, extrahepatic bile ducts, and pancreas. *Cancer* 1995; **75**: 171-190 [PMID: 8000995]
- 15 **Hundal R**, Shaffer EA. Gallbladder cancer: epidemiology and outcome. *Clin Epidemiol* 2014; **6**: 99-109 [PMID: 24634588 DOI: 10.2147/CLEP.S37357]
- 16 **Surveillance, Epidemiology and End-Results Program (SEER)**. The Four Most Common Cancers for Different Ethnic Populations 2013. Bethesda, MD: National Cancer Institute, 2013
- 17 **Miller G**, Jarnagin WR. Gallbladder carcinoma. *Eur J Surg Oncol* 2008; **34**: 306-312 [PMID: 17964753 DOI: 10.1016/j.ejso.2007.07.206]
- 18 **Inui K**, Yoshino J, Miyoshi H. Diagnosis of gallbladder tumors. *Intern Med* 2011; **50**: 1133-1136 [PMID: 21628925 DOI: 10.2169/internalmedicine.50.5255]
- 19 **Ministry of Internal Affairs and Communications**. Japan Statistical Yearbook, Chapter 21 Health and sanitation 21-15 Deaths and death rates by leading cause of death. Available from: URL: <http://www.stat.go.jp/english/data/tenkan/1431-21.htm>
- 20 **Murthy ND**, Rajaram D, Gautham MS, Shivraj NS, Pruthvish S,

- George PR, Mathew A. Trends in incidence of gallbladder cancer-Indian scenario. *Gastrointestinal Cancer: Targets and Therapy* 2011; **1**: 1-9
- 21 **Yamamoto M**, Nakajo S, Tahara E. Dysplasia of the gallbladder. Its histogenesis and correlation to gallbladder adenocarcinoma. *Pathol Res Pract* 1989; **185**: 454-460 [PMID: 2602218 DOI: 10.1016/S0344-0338(89)80062-7]
  - 22 **Sasatomi E**, Tokunaga O, Miyazaki K. Precancerous conditions of gallbladder carcinoma: overview of histopathologic characteristics and molecular genetic findings. *J Hepatobiliary Pancreat Surg* 2000; **7**: 556-567 [PMID: 11180887]
  - 23 **Albores-Saavedra JHD**. Atlas of tumor pathology, second series fascicle 22. In: (MD) neB, editor. Tumors of the gallbladder and extrahepatic biliary ducts: Armed Forces Institute of Pathology, 1986: 28-123
  - 24 **Johnstone AK**, Zuch RH, Anders KH. Oat cell carcinoma of the gallbladder. A rare and highly lethal neoplasm. *Arch Pathol Lab Med* 1993; **117**: 1009-1012 [PMID: 8215821]
  - 25 **Vaittinen E**. Carcinoma of the gall-bladder. A study of 390 cases diagnosed in Finland 1953-1967. *Ann Chir Gynaecol Fenn Suppl* 1970; **168**: 1-81 [PMID: 5268194]
  - 26 **Sumiyoshi K**, Nagai E, Chijiwa K, Nakayama F. Pathology of carcinoma of the gallbladder. *World J Surg* 1991; **15**: 315-321 [PMID: 1853609 DOI: 10.1007/BF01658722]
  - 27 **Duarte I**, Llanos O, Domke H, Harz C, Valdivieso V. Metaplasia and precursor lesions of gallbladder carcinoma. Frequency, distribution, and probability of detection in routine histologic samples. *Cancer* 1993; **72**: 1878-1884 [PMID: 8364865]
  - 28 **Roa I**, Araya JC, Villaseca M, De Aretxabala X, Riedemann P, Endoh K, Roa J. Preneoplastic lesions and gallbladder cancer: an estimate of the period required for progression. *Gastroenterology* 1996; **111**: 232-236 [PMID: 8698204]
  - 29 **Misra S**, Chaturvedi A, Misra NC, Sharma ID. Carcinoma of the gallbladder. *Lancet Oncol* 2003; **4**: 167-176 [PMID: 12623362 DOI: 10.1016/S1470-2045(03)01021-0]
  - 30 **Shukla VK**, Chauhan VS, Kumar M. Telomerase activation—one step on the road to carcinoma of the gall bladder. *Anticancer Res* 2006; **26**: 4761-4766 [PMID: 17214337]
  - 31 **Kumari N**, Corless CL, Warrick A, Beadling C, Nelson D, Neff T, Krishnani N, Kapoor VK. Mutation profiling in gallbladder cancer in Indian population. *Indian J Pathol Microbiol* 2014; **57**: 9-12 [PMID: 24739824 DOI: 10.4103/0377-4929.130849]
  - 32 **Kazmi HR**, Chandra A, Nigam J, Noushif M, Parmar D, Gupta V. Prognostic significance of K-ras codon 12 mutation in patients with resected gallbladder cancer. *Dig Surg* 2013; **30**: 233-239 [PMID: 23838952 DOI: 10.1159/000353133]
  - 33 **Deshpande V**, Nduaguba A, Zimmerman SM, Kehoe SM, Macconail LE, Lauwers GY, Ferrone C, Bardeesy N, Zhu AX, Hezel AF. Mutational profiling reveals PIK3CA mutations in gallbladder carcinoma. *BMC Cancer* 2011; **11**: 60 [PMID: 21303542 DOI: 10.1186/1471-2407-11-60]
  - 34 **Hezel AF**, Deshpande V, Zhu AX. Genetics of biliary tract cancers and emerging targeted therapies. *J Clin Oncol* 2010; **28**: 3531-3540 [PMID: 20547994 DOI: 10.1200/JCO.2009.27.4787]
  - 35 **Kim K**, Kim DH, Chae SW, Shin JH, Kim HJ, Do SI, Lee HJ, Koo JH, Pyo JS, Sohn JH. Expression of Cell Cycle-Related Proteins, p16, p53 and p63 as Important Prognostic Markers in Gallbladder Adenocarcinoma. *Pathol Oncol Res* 2013 Nov 1; Epub ahead of print [PMID: 24178677]
  - 36 **Ghosh M**, Sakhuja P, Singh S, Agarwal AK. p53 and beta-catenin expression in gallbladder tissues and correlation with tumor progression in gallbladder cancer. *Saudi J Gastroenterol* 2013; **19**: 34-39 [PMID: 23319036 DOI: 10.4103/1319-3767.105922]
  - 37 **Kuroki T**, Tajima Y, Matsuo K, Kanematsu T. Genetic alterations in gallbladder carcinoma. *Surg Today* 2005; **35**: 101-105 [PMID: 15674488 DOI: 10.1007/s00595-004-2906-2]
  - 38 **Xuan YH**, Choi YL, Shin YK, Kook MC, Chae SW, Park SM, Chae HB, Kim SH. An immunohistochemical study of the expression of cell-cycle-regulated proteins p53, cyclin D1, RB, p27, Ki67 and MSH2 in gallbladder carcinoma and its precursor lesions. *Histol Histopathol* 2005; **20**: 59-66 [PMID: 15578423]
  - 39 **Dixit R**, Shukla VK, Pandey M. Molecular alterations in gallbladder cancer. *World J Pathol* 2012; **1**: 31-34
  - 40 **Hart J**, Modan B, Hashomer T. Factors affecting survival of patients with gallbladder neoplasms. *Arch Intern Med* 1972; **129**: 931-934 [PMID: 4338212 DOI: 10.1001/archinte.129.6.931]
  - 41 **Inoue T**, Shiraki K, Fuke H, Yamanaka Y, Miyashita K, Ito K, Suzuki M, Sugimoto K, Murata K, Nakano T. Cardiac metastases of gallbladder carcinoma. *World J Gastroenterol* 2005; **11**: 2048-2049 [PMID: 15801006]
  - 42 **Misra S**, Chaturvedi A, Misra NC. Carcinoma gallbladder presenting with skeletal metastases. *Indian J Gastroenterol* 1997; **16**: 74 [PMID: 9114585]
  - 43 **Puneet R**, Kumar M, Khanna AK. Carcinoma gall bladder presenting as metastatic lung disease. *Indian J Gastroenterol* 1999; **18**: 44 [PMID: 10063760]
  - 44 **Ito M**, Mishima Y, Sato T. An anatomical study of the lymphatic drainage of the gallbladder. *Surg Radiol Anat* 1991; **13**: 89-104 [PMID: 1925922 DOI: 10.1007/BF01623880]
  - 45 **Matsumoto Y**, Fujii H, Aoyama H, Yamamoto M, Sugahara K, Suda K. Surgical treatment of primary carcinoma of the gallbladder based on the histologic analysis of 48 surgical specimens. *Am J Surg* 1992; **163**: 239-245 [PMID: 1739180 DOI: 10.1016/0002-9610(92)90109-5]
  - 46 **Jarnagin WR**, Ruo L, Little SA, Klimstra D, D'Angelica M, DeMatteo RP, Wagman R, Blumgart LH, Fong Y. Patterns of initial disease recurrence after resection of gallbladder carcinoma and hilar cholangiocarcinoma: implications for adjuvant therapeutic strategies. *Cancer* 2003; **98**: 1689-1700 [PMID: 14534886 DOI: 10.1002/cncr.11699]
  - 47 **Glenn F**, Reed C, Grafe WR. Biliary enteric fistula. *Surg Gynecol Obstet* 1981; **153**: 527-531 [PMID: 7280941]
  - 48 **Dwivedi AN**, Pandey M, Shukla RC, Shukla VK, Gaharwar S, Maurya BN. Biological behavior and disease pattern of carcinoma gallbladder shown on 64-slice CT scanner: a hospital-based retrospective observational study and our experience. *Indian J Cancer* 2012; **49**: 303-308 [PMID: 23238149 DOI: 10.4103/0019-509X.104496]
  - 49 **Arminski TC**. Primary carcinoma of the gallbladder; a collective review with the addition of 25 cases from the Grace Hospital, Detroit, Michigan. *Cancer* 1949; **2**: 379-398 [PMID: 18131399]
  - 50 **Fahim RB**, McDonald JR, Richards JC, Ferris DO. Carcinoma of the gallbladder: a study of its modes of spread. *Ann Surg* 1962; **156**: 114-124 [PMID: 13891308 DOI: 10.1097/0000658-196207000-00021]
  - 51 **Kapoor VK**, Pradeep R, Haribhakti SP, Sikora SS, Kaushik SP. Early carcinoma of the gallbladder: an elusive disease. *J Surg Oncol* 1996; **62**: 284-287 [PMID: 8691843]
  - 52 **Yoshimitsu K**, Honda H, Shinozaki K, Aibe H, Kuroiwa T, Irie H, Chijiwa K, Asayama Y, Masuda K. Helical CT of the local spread of carcinoma of the gallbladder: evaluation according to the TNM system in patients who underwent surgical resection. *AJR Am J Roentgenol* 2002; **179**: 423-428 [PMID: 12130444 DOI: 10.2214/ajr.179.2.1790423]
  - 53 **Grand D**, Horton KM, Fishman EK. CT of the gallbladder: spectrum of disease. *AJR Am J Roentgenol* 2004; **183**: 163-170 [PMID: 15208132 DOI: 10.2214/ajr.183.1.1830163]
  - 54 **Koo J**, Wong J, Cheng FC, Ong GB. Carcinoma of the gallbladder. *Br J Surg* 1981; **68**: 161-165 [PMID: 7470817 DOI: 10.1002/bjs.1800680307]
  - 55 **Liebowitz HR**. Primary carcinoma of the gall bladder. *Am J Digest Dis* 1939-1940; **6**: 381-387
  - 56 **Mattson H**. Carcinoma of the gall bladder; study of sixty cases. *Minnesota Med* 1942; **25**: 985-988
  - 57 **Rolleston HD**, Mcnee JW. Diseases of the liver, gall bladder and bile ducts. 3rd ed. London: The Macmillan co, 1929: 691

- 58 **Yochum TR**, Rowel J. Tumor and tumor like processes. In: Yochum TR, Rowel J, editors. Essentials of skeletal radiology. 1st ed. Baltimore, Maryland, USA: Williams and Wilkins, 1987: 975-1192
- 59 **Donohue JH**, Nagorney DM, Grant CS, Tsushima K, Ilstrup DM, Adson MA. Carcinoma of the gallbladder. Does radical resection improve outcome? *Arch Surg* 1990; **125**: 237-241 [PMID: 2302063 DOI: 10.1001/archsurg.1990.01410140115019]
- 60 AJCC Cancer Staging Handbook. 7th ed. New York: Springer-Verlag, 2010
- 61 **American Joint Committee on Cancer**. Gallbladder. In: Greene FL, Fleming ID, editors. AJCC Cancer Staging Manual. 6th ed. New York: Springer-Verlag, 2002
- 62 **White K**, Kraybill WG, Lopez MJ. Primary carcinoma of the gallbladder: TNM staging and prognosis. *J Surg Oncol* 1988; **39**: 251-255 [PMID: 3193769 DOI: 10.1002/jso.2930390407]
- 63 **Hederström E**, Forsberg L. Ultrasonography in carcinoma of the gallbladder. Diagnostic difficulties and pitfalls. *Acta Radiol* 1987; **28**: 715-718 [PMID: 2962605 DOI: 10.3109/02841858709177430]
- 64 **Tsuchiya Y**. Early carcinoma of the gallbladder: macroscopic features and US findings. *Radiology* 1991; **179**: 171-175 [PMID: 2006272]
- 65 **Hawkins WG**, DeMatteo RP, Jarnagin WR, Ben-Porat L, Blumgart LH, Fong Y. Jaundice predicts advanced disease and early mortality in patients with gallbladder cancer. *Ann Surg Oncol* 2004; **11**: 310-315 [PMID: 14993027 DOI: 10.1245/ASO.2004.03.011]
- 66 **Bach AM**, Loring LA, Hann LE, Illescas FF, Fong Y, Blumgart LH. Gallbladder cancer: can ultrasonography evaluate extent of disease? *J Ultrasound Med* 1998; **17**: 303-309 [PMID: 9586703]
- 67 **Nyland TG**, Hager DA, Herring DS. Sonography of the liver, gallbladder, and spleen. *Semin Vet Med Surg* (Small Anim) 1989; **4**: 13-31 [PMID: 2672213]
- 68 **Willard MD**, Dunstan RW, Faulkner J. Neuroendocrine carcinoma of the gallbladder in a dog. *J Am Vet Med Assoc* 1988; **192**: 926-928 [PMID: 3366681]
- 69 **Furlan A**, Ferris JV, Hosseinzadeh K, Borhani AA. Gallbladder carcinoma update: multimodality imaging evaluation, staging, and treatment options. *AJR Am J Roentgenol* 2008; **191**: 1440-1447 [PMID: 18941083 DOI: 10.2214/AJR.07.3599]
- 70 **Rodríguez-Fernández A**, Gómez-Río M, Medina-Benítez A, Moral JV, Ramos-Font C, Ramia-Angel JM, Llamas-Elvira JM, Ferrón-Orihuela JA, Lardelli-Claret P. Application of modern imaging methods in diagnosis of gallbladder cancer. *J Surg Oncol* 2006; **93**: 650-664 [PMID: 16724342 DOI: 10.1002/jso.20533]
- 71 **Franquet T**, Montes M, Ruiz de Azua Y, Jimenez FJ, Cozcolluela R. Primary gallbladder carcinoma: imaging findings in 50 patients with pathologic correlation. *Gastrointest Radiol* 1991; **16**: 143-148 [PMID: 2016028 DOI: 10.1007/BF01887330]
- 72 **Demachi H**, Matsui O, Hoshiba K, Kimura M, Miyata S, Kuroda Y, Konishi K, Tsuji M, Miwa A. Dynamic MRI using a surface coil in chronic cholecystitis and gallbladder carcinoma: radiologic and histopathologic correlation. *J Comput Assist Tomogr* 1997; **21**: 643-651 [PMID: 9216777 DOI: 10.1097/00004728-199707000-00025]
- 73 **Kuo YC**, Liu JY, Sheen IS, Yang CY, Lin DY, ChangChen CS. Ultrasonographic difficulties and pitfalls in diagnosing primary carcinoma of the gallbladder. *J Clin Ultrasound* 1990; **18**: 639-647 [PMID: 2172311]
- 74 **Bondetam S**. Sonographic diagnosis of primary carcinoma of the gallbladder. Summary of one year's examinations. *Diagn Imaging* 1981; **50**: 197-200 [PMID: 7297386]
- 75 **Wibbenmeyer LA**, Sharafuddin MJ, Wolverson MK, Heiberg EV, Wade TP, Shields JB. Sonographic diagnosis of unsuspected gallbladder cancer: imaging findings in comparison with benign gallbladder conditions. *AJR Am J Roentgenol* 1995; **165**: 1169-1174 [PMID: 7572497 DOI: 10.2214/ajr.165.5.7572497]
- 76 **Hattori M**, Inui K, Yoshino J, Miyoshi H, Okushima K, Nakamura Y, Naito T, Imaeda Y, Horibe Y, Hattori T, Nakazawa S. [Usefulness of contrast-enhanced ultrasonography in the differential diagnosis of polypoid gallbladder lesions]. *Nihon Shokakibyo Gakkai Zasshi* 2007; **104**: 790-798 [PMID: 17548945]
- 77 **Shukla VK**, Pandey M, Kumar M, Sood BP, Gupta A, Aryya NC, Shukla RC, Verma DN. Ultrasound-guided fine needle aspiration cytology of malignant gallbladder masses. *Acta Cytol* 1997; **41**: 1654-1658 [PMID: 9390120 DOI: 10.1159/000333156]
- 78 **Zargar SA**, Khuroo MS, Mahajan R, Jan GM, Shah P. US-guided fine-needle aspiration biopsy of gallbladder masses. *Radiology* 1991; **179**: 275-278 [PMID: 2006291]
- 79 **Jacobson BC**, Pitman MB, Brugge WR. EUS-guided FNA for the diagnosis of gallbladder masses. *Gastrointest Endosc* 2003; **57**: 251-254 [PMID: 12556797 DOI: 10.1067/mge.2003.86]
- 80 **Kimura K**. [Diagnosis for pedunculated polypoid lesions of the gallbladder by endoscopic ultrasonography]. *Nihon Shokakibyo Gakkai Zasshi* 1997; **94**: 249-260 [PMID: 9136581]
- 81 **Mitake M**, Nakazawa S, Naitoh Y, Kimoto E, Tsukamoto Y, Asai T, Yamao K, Inui K, Morita K, Hayashi Y. Endoscopic ultrasonography in diagnosis of the extent of gallbladder carcinoma. *Gastrointest Endosc* 1990; **36**: 562-566 [PMID: 2279643 DOI: 10.1016/S0016-5107(90)71164-9]
- 82 **Fujita N**, Noda Y, Kobayashi G, Kimura K, Yago A. Diagnosis of the depth of invasion of gallbladder carcinoma by EUS. *Gastrointest Endosc* 1999; **50**: 659-663 [PMID: 10536322 DOI: 10.1016/S0016-5107(99)80015-7]
- 83 **Haribhakti SP**, Kapoor VK, Gujral RB, Kaushik SP. Staging of carcinoma of the gallbladder--an ultrasonographic evaluation. *Hepatogastroenterology* 1997; **44**: 1240-1245 [PMID: 9356834]
- 84 **Aissi A**, Touri S. Ultrasonographic diagnosis gallbladder wall thickening. *The Internet Journal of Veterinary Medicine* 2008; **4**
- 85 **Varadarajulu S**, Eloubeidi MA. Endoscopic ultrasound-guided fine-needle aspiration in the evaluation of gallbladder masses. *Endoscopy* 2005; **37**: 751-754 [PMID: 16032495 DOI: 10.1055/s-2005-870161]
- 86 **Byrne MF**, Gerke H, Mitchell RM, Stiffler HL, McGrath K, Branch MS, Baillie J, Jowell PS. Yield of endoscopic ultrasound-guided fine-needle aspiration of bile duct lesions. *Endoscopy* 2004; **36**: 715-719 [PMID: 15280978 DOI: 10.1055/s-2004-825657]
- 87 **Yun EJ**, Cho SG, Park S, Park SW, Kim WH, Kim HJ, Suh CH. Gallbladder carcinoma and chronic cholecystitis: differentiation with two-phase spiral CT. *Abdom Imaging* 2004; **29**: 102-108 [PMID: 15160762 DOI: 10.1007/s00261-003-0080-4]
- 88 **Tsukada K**, Kurosaki I, Uchida K, Shirai Y, Ohashi Y, Yokoyama N, Watanabe H, Hatakeyama K. Lymph node spread from carcinoma of the gallbladder. *Cancer* 1997; **80**: 661-667 [PMID: 9264348]
- 89 **Kim BS**, Ha HK, Lee JJ, Kim JH, Eun HW, Bae IY, Kim AY, Kim TK, Kim MH, Lee SK, Kang W. Accuracy of CT in local staging of gallbladder carcinoma. *Acta Radiol* 2002; **43**: 71-76 [PMID: 11972466 DOI: 10.1080/028418502127347475]
- 90 **Kumaran V**, Gulati S, Paul B, Pande K, Sahni P, Chattopadhyay K. The role of dual-phase helical CT in assessing resectability of carcinoma of the gallbladder. *Eur Radiol* 2002; **12**: 1993-1999 [PMID: 12136317 DOI: 10.1007/s00330-002-1440-0]
- 91 **Furukawa H**, Kosuge T, Shimada K, Yamamoto J, Kanai Y, Mukai K, Iwata R, Ushio K. Small polypoid lesions of the gallbladder: differential diagnosis and surgical indications by helical computed tomography. *Arch Surg* 1998; **133**: 735-739 [PMID: 9688001 DOI: 10.1001/archsurg.133.7.735]
- 92 **Kumar A**, Aggarwal S. Carcinoma of the gallbladder: CT findings in 50 cases. *Abdom Imaging* 1994; **19**: 304-308 [PMID: 8075550 DOI: 10.1007/BF00198184]
- 93 **van Breda Vriesman AC**, Engelbrecht MR, Smithuis RH, Puylaert JB. Diffuse gallbladder wall thickening: differential diagnosis. *AJR Am J Roentgenol* 2007; **188**: 495-501 [PMID: 17242260 DOI: 10.2214/AJR.05.1712]
- 94 **Ben Farhat L**, Askri A, Jeribi R, Daly N, Hendaoui L. [CT evaluation of locoregional spread of carcinoma of the gallbladder]. *J Chir* (Paris) 2009; **146**: 34-39 [PMID: 19446691 DOI: 10.1016/



- j.jchir.2009.02.005]
- 95 **Oikarinen H.** Diagnostic imaging of carcinomas of the gallbladder and the bile ducts. *Acta Radiol* 2006; **47**: 345-358 [PMID: 16739693 DOI: 10.1080/02841850600580317]
- 96 **Liang JL,** Chen MC, Huang HY, Ng SH, Sheen-Chen SM, Liu PP, Kung CT, Ko SF. Gallbladder carcinoma manifesting as acute cholecystitis: clinical and computed tomographic features. *Surgery* 2009; **146**: 861-868 [PMID: 19744453 DOI: 10.1016/j.surg.2009.04.037]
- 97 **Lee TY,** Ko SF, Huang CC, Ng SH, Liang JL, Huang HY, Chen MC, Sheen-Chen SM. Intraluminal versus infiltrating gallbladder carcinoma: clinical presentation, ultrasound and computed tomography. *World J Gastroenterol* 2009; **15**: 5662-5668 [PMID: 19960562 DOI: 10.3748/wjg.15.5662]
- 98 **Itai Y.** Computed tomographic evaluation of gallbladder disease. *Crit Rev Diagn Imaging* 1987; **27**: 113-152 [PMID: 3301215]
- 99 **Kim JH,** Kim TK, Eun HW, Kim BS, Lee MG, Kim PN, Ha HK. Preoperative evaluation of gallbladder carcinoma: efficacy of combined use of MR imaging, MR cholangiography, and contrast-enhanced dual-phase three-dimensional MR angiography. *J Magn Reson Imaging* 2002; **16**: 676-684 [PMID: 12451581 DOI: 10.1002/jmri.10212]
- 100 **Oberoi RJA,** Kumar R, Pandey P, Pandey K. MRI in preoperative evaluation of GBC. *Asian Oceanian Journal of Radiology* 2004; **9**: 57-63
- 101 **Ramos-Font C,** Gómez Río M, Rodríguez-Fernández A, Sánchez Sánchez R, Llamas Elvira JM. [Positron tomography with 18F-fluorodeoxyglucose in the preoperative evaluation of gall bladder lesions suspicious of malignancy. Diagnostic utility and clinical impact]. *Rev Esp Med Nucl* 2011; **30**: 267-275 [PMID: 21612846 DOI: 10.1016/j.rem.2011.02.004]
- 102 **Karim ME,** Bassam AW, Michele M. Advances in diagnosis, treatment and palliation of gallbladder cancer. *AcademyPublish.org-Journal of Medical Research and Science* 2012; **2**: 92
- 103 **Kim SJ,** Lee JM, Lee JY, Choi JY, Kim SH, Han JK, Choi BI. Accuracy of preoperative T-staging of gallbladder carcinoma using MDCT. *AJR Am J Roentgenol* 2008; **190**: 74-80 [PMID: 18094296 DOI: 10.2214/AJR.07.2348]
- 104 **Kalra N,** Suri S, Gupta R, Natarajan SK, Khandelwal N, Wig JD, Joshi K. MDCT in the staging of gallbladder carcinoma. *AJR Am J Roentgenol* 2006; **186**: 758-762 [PMID: 16498103 DOI: 10.2214/AJR.04.1342]
- 105 **Koh T,** Taniguchi H, Yamaguchi A, Kunishima S, Yamagishi H. Differential diagnosis of gallbladder cancer using positron emission tomography with fluorine-18-labeled fluoro-deoxyglucose (FDG-PET). *J Surg Oncol* 2003; **84**: 74-81 [PMID: 14502780 DOI: 10.1002/jso.10295]
- 106 **Corvera CU,** Blumgart LH, Akhurst T, DeMatteo RP, D'Angelica M, Fong Y, Jarnagin WR. 18F-fluorodeoxyglucose positron emission tomography influences management decisions in patients with biliary cancer. *J Am Coll Surg* 2008; **206**: 57-65 [PMID: 18155569 DOI: 10.1016/j.jamcollsurg.2007.07.002]
- 107 **Greene FL,** Page DL, Fleming ID, Fritz A, Balch CM, Haller DG. AJCC cancerstaging manual. 6th ed. New York, NY: Springer, 2002: 139-142
- 108 **Otero JC,** Proske A, Vallilengua C, Luján M, Poletto L, Otero JR, Pezzotto SM, Celoria G. Gallbladder carcinoma: intraoperative imprint cytology, a helpful and valuable screening procedure. *J Hepatobiliary Pancreat Surg* 2008; **15**: 157-160 [PMID: 18392708 DOI: 10.1007/s00534-007-1253-2]
- 109 **Iqbal M,** Gondal KM, Qureshi AU, Tayyab M. Comparative study of ultrasound guided fine needle aspiration cytology with open/laparoscopic biopsy for diagnosis of carcinoma gallbladder. *J Coll Physicians Surg Pak* 2009; **19**: 17-20 [PMID: 19149974 DOI: 10.2009/JCPS.1720]
- 110 **Meera RS,** Jhala D, Eloubeidi MA, Eltoum I, Chhieng DC, Crowe DR, Varadarajulu S, Jhala N. Endoscopic ultrasound-guided FNA biopsy of bile duct and gallbladder: analysis of 53 cases. *Cytopathology* 2006; **17**: 42-49 [PMID: 16417564 DOI: 10.1111/j.1365-2303.2006.00319.x]
- 111 **Shukla VK,** Gurubachan D, Dixit VK. Diagnostic value of serum CA242, CA 19-9, CA 15-3 and CA 125 in patients with carcinoma of the gallbladder. *Trop Gastroenterol* 2006; **27**: 160-165 [PMID: 17542293]
- 112 **Bartlett DLFY.** Tumors of the gallbladder. In: Blilmgart LHFY, editor. Surgery of the liver and biliary tract. 3rd ed. London: WB Saunders, 2000: 993-1015
- 113 **Bernades P,** Schlegel N, Potet F, Menaché D. [Cancer of the gallbladder with alpha-feto-protein. Disappearance of the protein after exeresis of the tumor]. *Nouv Presse Med* 1972; **1**: 1297-1298 [PMID: 5026586]
- 114 **Shukla VK,** Goel S, Trigun SK, Sharma D. Electrophoretic pattern of proteins in carcinoma of the gallbladder. *Eur J Cancer Prev* 2008; **17**: 9-12 [PMID: 18090904 DOI: 10.1097/CEJ.0b013e3280145e1b]
- 115 **Wang J,** Zhang J, Wu W, Duan X, Wang S, Zhang M, Zhou S, Mo F, Xu Y, Shi J, Wu J. Evaluation of gallbladder lipid level during carcinogenesis by an infrared spectroscopic method. *Dig Dis Sci* 2010; **55**: 2670-2675 [PMID: 19957036 DOI: 10.1007/s10620-009-1045-4]
- 116 **Kang CM,** Choi GH, Park SH, Kim KS, Choi JS, Lee WJ, Kim BR. Laparoscopic cholecystectomy only could be an appropriate treatment for selected clinical R0 gallbladder carcinoma. *Surg Endosc* 2007; **21**: 1582-1587 [PMID: 17479340 DOI: 10.1007/s00464-006-9133-4]
- 117 **Mekeel KL,** Hemming AW. Surgical management of gallbladder carcinoma: a review. *J Gastrointest Surg* 2007; **11**: 1188-1193 [PMID: 17712596 DOI: 10.1007/s11605-007-0115-1]
- 118 **Shirai Y,** Yoshida K, Tsukada K, Muto T, Watanabe H. Early carcinoma of the gallbladder. *Eur J Surg* 1992; **158**: 545-548 [PMID: 1360827]
- 119 **Wakai T,** Shirai Y, Yokoyama N, Nagakura S, Watanabe H, Hatakeyama K. Early gallbladder carcinoma does not warrant radical resection. *Br J Surg* 2001; **88**: 675-678 [PMID: 11350438 DOI: 10.1046/j.1365-2168.2001.01749.x]
- 120 **Shimizu Y,** Ohtsuka M, Ito H, Kimura F, Shimizu H, Togawa A, Yoshidome H, Kato A, Miyazaki M. Should the extrahepatic bile duct be resected for locally advanced gallbladder cancer? *Surgery* 2004; **136**: 1012-1017; discussion 1018 [PMID: 15523394 DOI: 10.1016/j.surg.2004.04.032]
- 121 **Andrén-Sandberg A.** Diagnosis and management of gallbladder cancer. *N Am J Med Sci* 2012; **4**: 293-299 [PMID: 22866265 DOI: 10.4103/1947-2714.98586]
- 122 **Gourgiotis S,** Kocher HM, Solaini L, Yarollahi A, Tsiambas E, Salemis NS. Gallbladder cancer. *Am J Surg* 2008; **196**: 252-264 [PMID: 18466866 DOI: 10.1016/j.amjsurg.2007.11.011]
- 123 **Reid KM,** Ramos-De la Medina A, Donohue JH. Diagnosis and surgical management of gallbladder cancer: a review. *J Gastrointest Surg* 2007; **11**: 671-681 [PMID: 17468929 DOI: 10.1007/s11605-006-0075-x]
- 124 **Mojica P,** Smith D, Ellenhorn J. Adjuvant radiation therapy is associated with improved survival for gallbladder carcinoma with regional metastatic disease. *J Surg Oncol* 2007; **96**: 8-13 [PMID: 17516546 DOI: 10.1002/jso.20831]
- 125 **Malik IA,** Aziz Z, Zaidi SH, Sethuraman G. Gemcitabine and Cisplatin is a highly effective combination chemotherapy in patients with advanced cancer of the gallbladder. *Am J Clin Oncol* 2003; **26**: 174-177 [PMID: 12714891 DOI: 10.1097/01.COC.0000018037.59196.67]
- 126 **Julka PK,** Puri T, Rath GK. A phase II study of gemcitabine and carboplatin combination chemotherapy in gallbladder carcinoma. *Hepatobiliary Pancreat Dis Int* 2006; **5**: 110-114 [PMID: 16481295]
- 127 **Sharma A,** Mohanti B, Raina V, Shukla N, Pal S, Dwary A, Deo S, Sahni P, Garg P, Thulkar S, DattaGupta S, Rath G. A phase II study of gemcitabine and oxaliplatin (Oxigem) in unresectable gall bladder cancer. *Cancer Chemother Pharmacol* 2010; **65**: 497-502 [PMID: 19575200 DOI: 10.1007/s00280-009-1055-0]
- 128 **Zhu AX,** Hong TS, Hezel AF, Kooby DA. Current management



of gallbladder carcinoma. *Oncologist* 2010; **15**: 168-181 [PMID: 20147507 DOI: 10.1634/theoncologist.2009-0302]

129 **Hu YP**, Tan ZJ, Wu XS, Liu TY, Jiang L, Bao RF, Shu YJ, Li ML,

Weng H, Ding Q, Tao F, Liu YB. Triptolide induces s phase arrest and apoptosis in gallbladder cancer cells. *Molecules* 2014; **19**: 2612-2628 [PMID: 24566325 DOI: 10.3390/molecules19022612]

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## Fluoroscopy guided percutaneous renal access in prone position

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most crucial step of this procedure. A proper access is the gateway to success. However, this crucial step has the steepest learning curve for, in a fluoroscopy guided access, it involves visualizing a three dimensional anatomy on a two dimensional fluoroscopy screen. This review describes the anatomical basis of the renal access. It provides a literature review of all aspects of percutaneous renal access along with the advances that have taken place in this field over the years. The article describes a technique to determine the site of skin puncture, the angle and depth of puncture using a simple mathematical principle. It also reviews the common problems faced during the process of puncture and dilatation and describes the ways to overcome them. The aim of this article is to provide the reader a step by step guide for percutaneous renal access.

**Key words:** Fluoroscopy; Percutaneous renal access; Percutaneous nephrolithotomy; Learning curve; Kidney

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**Core tip:** This article is a review of the various fluoroscopic guided renal access techniques. It provides an in depth description of the technique with the aim that the urologist can have a step by step guide of the procedure. It gives an anatomical basis of percutaneous renal access and gives description of determining the skin site, angle and depth of puncture. It also describes the difficulties faced and incorporates suggestions to prevent and overcome them.

### Abstract

Percutaneous nephrolithotomy is a very commonly done procedure for management of renal calculus disease. Establishing a good access is the first and probably the

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## INTRODUCTION

Rupel and Brown first reported Percutaneous nephrolithotomy in 1941<sup>[1]</sup>. Goodwin *et al.*<sup>[2]</sup> described percutaneous trocar nephrostomy in a hydronephrotic kidney in 1955. The technique gained popularity especially after the description by Fernstrom and Johansson in 1976<sup>[3-5]</sup>. The improvement in endourological equipment and the advancement in techniques resulted in percutaneous nephrolithotomy (PCNL) getting accepted as the gold standard for the treatment of patients with renal stones larger than 20 mm in diameter<sup>[6]</sup>. Its popularity and acceptance amongst urologists and patients is largely due to the fact that it is minimally invasive and is associated with low morbidity<sup>[7]</sup>. Initially the procedure was done only in prone position using fluoroscopy guidance. However, in the last couple of decades use of ultrasonography alone or along with fluoroscopy has been used for percutaneous renal access<sup>[8]</sup>. Various modifications in the position of patient have also been described to overcome some limitations and drawbacks of the percutaneous renal access in prone position<sup>[9]</sup>. Despite these changes fluoroscopy guided access in prone position is still the most commonly used technique for PCNL<sup>[10]</sup>. The prone position is associated with a significantly shorter nephrostomy tract length and more potential access sites, which may improve ease and safety of percutaneous renal access<sup>[11]</sup>. In European countries, urologist establishes their own percutaneous renal access, but in the United States, access is often performed by interventional radiologists. Studies have shown lower stone free rate and a higher complication rate in radiologist performed renal access<sup>[12-14]</sup>. Despite these facts and the documented safety and efficacy of urologist acquired percutaneous renal access, as few as 11% of urologists who perform PCNL achieve access<sup>[15]</sup>. This low success rate is attributed to probably a lack of skill<sup>[16]</sup>. This is probably due to the difficulty in visualizing and mentally imbibing the three dimensional anatomy of the pelvicalyceal system on the two dimensional fluoroscopy screen<sup>[17]</sup>. The purpose of this review is to describe the various aspects of the technique of fluoroscopy guided percutaneous renal access in prone position. It is the attempt of the authors to provide a step by step guide of all aspects of the technique. Finally, the authors describe their technique in detail and the rationale behind it.

## INSERTION OF URETERIC CATHETER

At the beginning of the procedure, a 5 Fr or 6 Fr ureteric catheter is inserted in the collecting system either using a rigid cystoscope (with the patient in lithotomy position) or a flexible cystoscope (with the patient prone). This is used to instill contrast to opacify the system. Also it can be used to flush saline so as to distend the system, flush small gravels during the process of stone fragmentation and at times to pass a

glide wire for insertion of a double J (DJ) stent, at the end of the procedure. A Foley catheter is also passed by the side of the ureteric catheter. Both the catheters are secured with each other to prevent inadvertent slipping out of the ureteric catheter.

## PRONE POSITIONING

This is an important maneuver which, if not done properly, can result in potentially serious injury to the patient. The ideal way would be to have the patient supine, have a separate trolley by the side of the operating table, shift the patient in supine position to the side trolley, remove the monitoring devices and then make the patient prone on the operating table and immediately connect the devices which have been disengaged or removed. These maneuvers should be done with adequate staff and with proper co-ordination between the anesthesiologist managing the airway, endotracheal tube and neck and the staff managing the chest and torso. Although cervical spine injury during prone positioning under anesthesia is rare, it has been reported with both over flexion and over extension during prolonged procedures<sup>[18]</sup>. Patients with cervical spine pathology, Down's syndrome or rheumatoid arthritis or patients with myelopathic syndromes are at the greatest risk. Post operative visual loss is an uncommon (0.2% of spinal surgeries in one review) but grave complication of prone surgery<sup>[19]</sup>. The ability to maintain good ventilation along with the hemodynamic stability throughout the procedure is the challenge which the anesthesiologists face. For a healthy, adequately anesthetized patient, these may be clinically insignificant; however, for those with associated co morbidities, it can be very precarious. Hence, irrespective of whether the procedure is done under general or regional anesthesia, constant vigilant monitoring during the procedure is a must. Anesthesia for PCNL cannot and should not be taken lightly.

## POSITION OF PATIENT

Care should be taken to ensure that the pressure points are properly padded and limbs are positioned in a way that undue stretch, especially of the joints is avoided. This would prevent inadvertent injury and stretch of the nerves. The chest and abdomen is supported in a way to ensure free movements for the pulmonary capacity is greater in the prone compared to the supine position<sup>[18]</sup>. After positioning the flank is properly prepared and the unsterile areas covered with drapes (Figure 1).

## ARRANGEMENT OF TROLLEYS

This is as shown in the figure. This helps to have a clear view of the fluoroscopy and endo camera monitors (Figure 2).



Figure 1 Position of patient.

## INSTILLATION OF CONTRAST

Contrast is instilled *via* the ureteric catheter to opacify the pelvicalyceal system and identify the calyx which should be punctured. The contrast should be diluted in ratio of 1:3. The ureteric catheter, if placed in the upper pole, should be pulled down a bit so that it is in the pelvis. This helps in proper filling of all the calyces. The contrast should be instilled slowly to prevent extravasation. There should be continuous fluoroscopy monitoring so that which calyces are filled earlier and which later can be seen and this helps to identify the posterior calyx.

## WHICH POLE TO PUNCTURE?

The creation of a proper percutaneous renal access is the gateway to success or disaster in PCNL<sup>[10,20,21]</sup>. A basic understanding of anatomy is needed to plan this. The kidneys lie on the posterior abdominal wall against the psoas muscle with their longitudinal axis parallel to the oblique course of the psoas at an angle of 13° to 30° to the midline. Also, as the psoas major muscle is cone shaped, the kidneys, in their longitudinal axis have a dorsal tilt with the superior poles being more medial and more posterior than the inferior poles. As the hilar region is rotated anteriorly on the psoas muscle, the kidneys are rotated about 30° posteriorly and hence the lateral aspect of the kidney is posterior to the medial aspect. The kidneys are also angled 30°-50° behind the frontal (coronal) plane with the lower pole anterior to the upper pole<sup>[22]</sup>. In prone position, the pelvis tends to fall anteriorly on the psoas muscle; hence the lower pole, pelvis and the proximal end of the ureter are placed more anteriorly than the upper pole<sup>[23,24]</sup>. The calyceal drainage of poles of the kidneys is also very important. Sampaio found that, in the cases he studied, the superior pole was drained by only one midline calyceal infundibulum in 98.6% of cases; the inferior pole was drained by paired calyces arranged in two rows in 58% and by a single mid line calyceal infundibulum in 42% of cases and the mid pole was drained by paired calyces arranged in two rows (anterior and posterior) in 96% of cases<sup>[22]</sup>. This

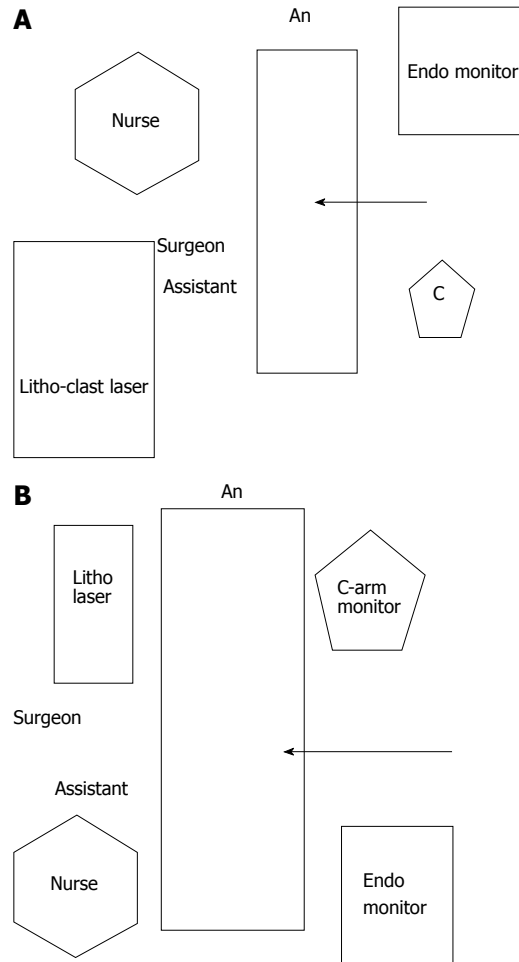
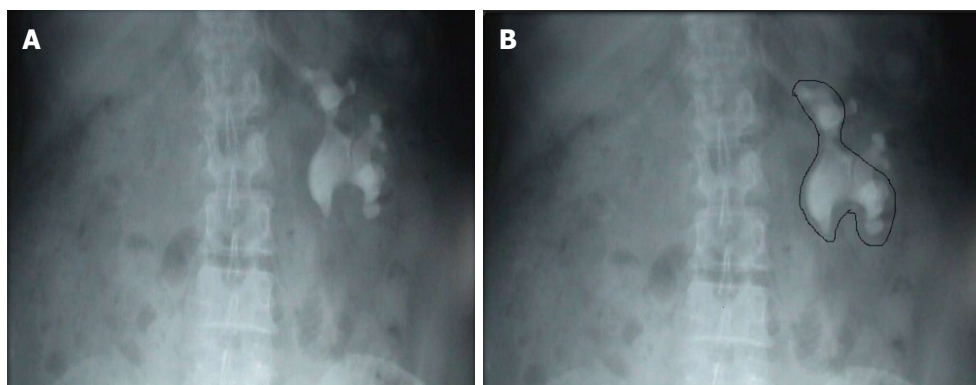


Figure 2 Arrangement of trolleys. A: Arrangement for lower pole puncture; B: Arrangement for upper pole puncture.

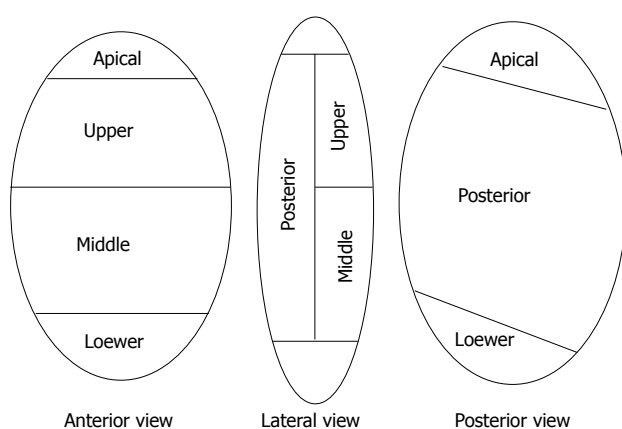
has important implications for percutaneous renal access as it will be easier to access endoscopically a polar region drained by a single infundibulum, which usually has suitable diameter, rather than a polar region drained by paired calyces. He also found that for best access to the pelvic-ureteric junction (PUJ) one should choose a pole whose calyx forms an angle of 90° or more with the PUJ<sup>[22]</sup>.

The planning for puncture begins preoperatively by proper assessment of the imaging studies. Traditionally intravenous urography was used for functional and anatomical assessment of the collecting system. Nowadays, CT urography with coronal reconstruction is getting popular<sup>[25]</sup>. The advantages of the CT scan over intravenous urography is the ability to assess the spatial relationship of the kidney relative to the stone, depict the calyceal anatomy in 3D format to choose the access site, assess risk of pleural or bowel injury and even predict success of a sub costal fluoroscopic access for upper pole puncture. The preoperative detection of heptosplenomegaly or the presence of retro renal colon allows serious complications related to tract placement to be avoided<sup>[26-29]</sup>. These advantages are offset by the slightly higher cost and lack of widespread availability of multiplanar CT scans in





**Figure 3 Outline-o-gram.** A: KUB showing a Left staghorn calculus; B: Outline-o-gram suggests that most of the calculus can be cleared by the lower puncture and a separate puncture may be needed for the residual fragment.



**Figure 4 Arterial blood supply of kidney.**

developing countries.

Whichever imaging modality is used the urologist has to select a pole for puncture which provides the straightest path along the stone axis and would provide maximum or complete stone clearance. A useful adjunct to make this decision would be to make an "outline-o-gram". As shown in Figure 3A there is a complete stag horn calculus. The "outline-o-gram" as shown in Figure 3B indicates that most of the calculus can be cleared by a lower pole puncture. The mid pole calculus would need a separate puncture or use of a flexible nephroscope. Thus an "outline-o-gram" can serve as a guide to determine which pole to puncture and also to decide whether multiple tracts will be needed.

### WHICH CALYX TO PUNCTURE?

The literature is clear about the fact that it should always be the posterior calyx which should be punctured for a safe and complication free access<sup>[20,21]</sup>.

### WHY TO PUNCTURE THE POSTERIOR CALYX?

A good understanding of the renal anatomy provides

answer to this question. The kidney gets its blood supply from the renal artery which divides into the anterior and posterior branch. These further divide into segmental arteries which supply specific areas of the kidney as shown in the figure (Figure 4). As these arteries are end arteries, there is a zone of relative avascularity between these two divisions, called as the Brodel's line of bloodless incision. The potential for bleeding complications is the least in this area. Due to the renal rotation, the posterior calices are usually oriented with their long axis pointing towards the Brodel's line. Hence puncture of a posterior calyx will traverse this relatively avascular zone<sup>[30]</sup>. Also, as the patient is prone, it will provide the direct path to the renal pelvis. If an anterior calyx is punctured, there is increased risk of bleeding as it does not traverse through the Brodel's line. More parenchyma is traversed to reach the calyx, resulting in more renal damage. Also, as there will be an acute angle between the line of puncture and the infundibulum, entry in the renal pelvis will be difficult, associated with more torque and thus increased bleeding and damage to the renal parenchyma<sup>[20,21]</sup>.

### CALYCEAL ORIENTATION-WHICH CALYX IS THE POSTERIOR CALYX?

The renal papillae drain into the minor calyces which may be simple or compound. There are three drainage zones, upper, middle and the lower pole. Compound calyces are the rule in upper pole, are common in the lower pole and are rare in the middle pole.

Investigators have attempted to differentiate calyces as anterior or posterior solely on the basis of their medial or lateral orientation as seen on IVU. The available anatomical references on this aspect are contradictory, confusing and incomplete. In 1901, Brodel studied corrosion casts of 70 cadaveric kidneys. He depicted the anterior calyces as medial and posterior as lateral<sup>[31]</sup>. Hodson, in 1972, described exactly the opposite, *i.e.*, the anterior calyces located laterally and posterior calyces located medially<sup>[32]</sup>. Then, in 1984,

Kaye and Reinke<sup>[33]</sup> measured calyceal angles from the axial CT images. They concluded that the Brodel pattern is seen in 69% of right kidneys while 70% of left kidneys have a Hodson pattern<sup>[33]</sup>. Sampaio *et al*<sup>[34,35]</sup> studied 140 endocasts and found that the anterior calyces are lateral in 28%, posterior calyces are lateral in 19%, and in 53% endocasts the anterior and posterior calyces had varied positions, superimposed or alternately distributed (in one region the most lateral were the anterior calyces and in another the posterior calyces)<sup>[34,35]</sup>. He found that the calyceal orientation was region dependent. The typical anterior and posterior pattern of the calyces is seen only in the middle pole<sup>[22]</sup>. The lower pole has this arrangement in only 58% cases while the upper pole almost uniformly has a compound calyceal system<sup>[22]</sup>. This implies that in the lower and upper pole the calyces are dominantly oriented in the direction of their respective poles. This has been further studied by 3D CT renderings which have also looked at the primary plane of the calyceal group. Miller *et al*<sup>[17]</sup> found that in the upper pole the primary plane of the calyces in the upper pole was Medial/Lateral and generally neutral relative to the anteroposterior axis of the kidney. As the upper pole is more posterior in the prone position, access *via* any calyx would provide a working tract that parallels the longitudinal axis of the kidney. This would mean access *via* an angle which would allow rigid instruments to reach most of the calyces in the kidney<sup>[17]</sup>. However, preferably the lateral most calyx should be punctured in the upper pole as puncturing a medial calyx is associated with significant risk of causing injury to the posterior segmental artery<sup>[36]</sup>. Eisner *et al*<sup>[37]</sup> studied the lower pole anatomy by CT scans in 101 units. They found that if there were two calices in the lower pole, the medial calyx was anterior in 95% of units while the lateral calyx was posterior in 93% of units. If there were 3 calices in the lower pole, than the medial most calyx was anterior in 93 of units. In such renal units the lateral to most medial, *i.e.*, the second calyx was posterior in 70% of units while the lateral most calyx was anterior in 71% of units. In 31% of cases, no calyx was truly posterior. In these kidneys, though both the calices were anterior, one of the calices was less anterior than the other. Their study showed that regardless of the number of lower pole calices, the most medial calyx on two dimensional imaging is anteriorly facing 94% of times. They recommended that the calyx, just lateral to the medial calyx, the second calyx, is statistically the most likely to be posterior facing and the most posterior positioned calyx<sup>[37]</sup>.

## HOW TO IDENTIFY THE POSTERIOR CALYX ON FLUOROSCOPY?

On account of the unreliability of the antero-posterior radiography to determine the optimal posterior calyx for entry additional maneuvers are needed<sup>[35]</sup>. With

the patient in prone position, diluted contrast when instilled will fill the dependent anterior calices first. Thus the posterior calices will be filled later and would appear less dense<sup>[21]</sup>. Injection of 5-10 mL of air *via* the ureteric catheter also helps to identify the posterior calices as air will preferentially enter these calices when the patient is prone<sup>[21,38]</sup>. Despite these maneuvers if there is dilemma in indentifying the posterior calyx, movement of the C arm can help to identify the posterior calyx. In the prone position, the posterior calyces move in the opposite direction to the image intensifier on the C arm. If the C arm is rotated towards the surgeon then the posterior calices move away and shorten. *Vice versa*, if the C arm is rotated away from the surgeon then the posterior calices appear elongated. Thus by moving the C arm way from the surgeon one can identify the laterally placed calices as posterior and by moving the C arm towards the surgeon the posterior calices appear more medially placed and appear end on<sup>[9]</sup>.

## NEEDLE USED FOR PUNCTURE

A diamond tip needle and not a bevel tip needle should be used for puncture. A diamond tip needle has symmetrical tip which exerts equal force in all directions on the tissue. Hence the tissue is cut in the moving direction of the needle tip. A bevel-tip needle exerts forces asymmetrically so cutting of the tissue occurs at an offset angle depending on the bevel angle, needle flexibility and tissue properties<sup>[39]</sup>. The size of the needle used for puncture is a matter of debate. The options are a 21 gauge needle (which allows a 0.018 inch guide wire) or an 18 gauge needle (which allows a 0.035 inch guide wire). The 18 gauge needle is stiffer but more traumatic. The 21 gauge needle is less traumatic but less stiff and hence cannot maintain the trajectory adequately. Also, the 0.018 inch guide wire that passes through the 21 gauge needle must be exchanged for a standard 0.035 inch guide wire for subsequent tract dilatation. This requires an extra step, which adds to the complexity of the procedure and increases the risk of loss of access. Weighing the pros and cons of both it would be rational to use the 21 gauge needle when the surgeon is less experienced or if minimizing trauma is the need of the moment. The 18 gauge needle should be used by an experienced surgeon who is confident of attaining access with minimum attempts<sup>[40]</sup>.

## WHAT SHOULD BE THE TRAJECTORY OF THE NEEDLE?

Renal pelvis should not be punctured directly as there is very high risk of injuring a retro pelvic vessel (artery and/or vein). Studies by Sampaio have proved beyond doubt that puncture through the infundibulum of a calyx is associated with a significant risk of significant

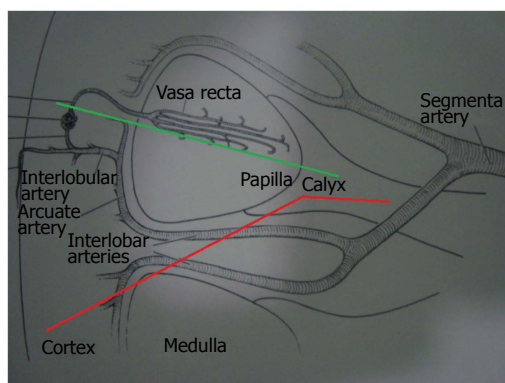


Figure 5 Trajectory of needle during puncture.

bleeding from interlobar vessels. There is an added risk of through and through puncture of the collecting system. The risk of injury to a major arterial vessel is maximum in the upper pole where puncture of the upper pole infundibulum puncture may cause damage to posterior segmental artery, which is related to the posterior surface of upper pole infundibulum in 57% of cases. Damage to this artery may lead to loss of upto 50% of the renal parenchyma as well as serious hemorrhage<sup>[30,35]</sup>.

The trajectory of the needle during puncture should be such that it aims at the fornix and not at the infundibula (Figure 5). In other words we should aim for the center of the calyx posterolaterally *via* the renal parenchyma. When puncture is made through a fornix, no arterial injury occurs and venous injury occurs in less than 8% cases<sup>[30]</sup>.

## CRITERIA FOR A GOOD PUNCTURE

Percutaneous renal access through a calyx must meet five conditions that guarantee safe access and avoids complications<sup>[41]</sup>: (1) Access should be performed from a posterolateral aspect; (2) Access should be through the renal parenchyma; (3) Access should be towards the center of a calyx posterolaterally; (4) Access should be towards the center of the renal pelvis and as a result of these four conditions; and (5) The trajectory does not damage any major blood vessels.

## TYPES OF FLUOROSCOPY GUIDED PUNCTURE

There are two types of fluoroscopy guided puncture techniques-the Bull's eye and the Triangulation Technique. Besides these two there are a number of variations described<sup>[42]</sup>.

### BULL'S EYE TECHNIQUE

It is also called the Eye of the Needle technique<sup>[20]</sup>. The target calyx is identified with the C arm at 0° in the axial plane. Then the C arm is rotated 30° towards the

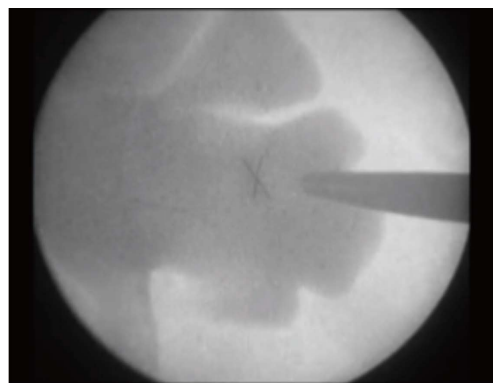


Figure 6 Bull's eye appearance of the needle.

surgeon and the calyx to be punctured would appear end on the fluoroscopy screen. A tilt of 5°-10° in the caudal direction for the lower pole or in the cranial direction for the upper pole may be necessitated to have a circular end on appearance of the target posterior calyx<sup>[21]</sup>. The position on the skin overlying the selected calyx is then marked and the puncture initiated. The needle is advanced at the end of full-expiration. It is seen as a Bull's eye (as a dot) on the fluoroscopy screen. If a longitudinal segment of needle is seen then it indicates that the trajectory is not correct and adjustment needs to be made accordingly. The C arm may be rotated by few degrees away from the surgeon to get a proper perspective of the depth of the puncture. The needle will now be seen in profile. It is then advanced to puncture the calyx. Free efflux of urine confirms the position in the collecting system<sup>[20,21,41,42]</sup>. To minimize radiation to the hands, the needle could be held with hemostat, sponge forceps or a purpose-built radiolucent needle holder (Figure 6).

### Modifications of the bull's eye technique

Bilen *et al*<sup>[43]</sup>, described the use of an in-line laser pointer to guide renal access in which the laser was attached within the field of the receiving head of the C-arm fluoroscopy unit. Ko *et al*<sup>[44]</sup> described a further modification using a C arm mounted laser positioning device where the laser beam is focused on the hub of the needle continuously so that the correct alignment is maintained during the puncture without the use of fluoroscopy. This approach may reduce fluoroscopic exposure early in the learning curve. As experience increased the muscle memory leads to maintenance of the correct needle alignment<sup>[42]</sup>.

## TRIANGULATION TECHNIQUE

Triangulation technique is the technique of using two known points of reference to locate a third unknown point. It is guided by biplanar fluoroscopy. The medial and lateral plane is assessed with the C arm at 0°. The depth is assessed by rotating the C arm in the

cranial or caudal direction by 30°. The target calyx is identified with the C arm at 0°. Then the line of puncture is aligned with the infundibulum. With the C arm at 0° the needle is introduced through the skin incision. The left and right, *i.e.*, the mediolateral adjustments are made and the needle is aligned with calyx. Then the C arm is rotated 30°, towards the head end for lower pole punctures and towards the foot end for upper pole punctures. The needle is then oriented in the up and down, *i.e.*, the cephalo-caudal position so that the orientation is again towards the desired calyx. When making the adjustments in one plane it is necessary to maintain the orientation of the needle in the other plane. The needle is then advanced with the C arm tilted 30° to give an idea regarding the depth and the respiration suspended at end expiration. After advancing the needle for several centimeters shift the C arm to 0° to see that the trajectory of the needle is still properly aligned to the target calyx in the mediolateral plane. If necessary the needle trajectory can be readjusted to maintain proper targeting. It is imperative that to minimize trauma to the renal parenchyma, the adjustment of the needle plane should be done when the needle is outside the renal capsule and not when the needle is in the parenchyma. A slight jiggle of the needle causing indentation of the desired calyx is a further sign that the trajectory of the needle is correct. If the needle position in the medial-lateral and cephalo-caudal planes is maintained, the needle should enter the targeted calyx<sup>[20,24,39-41]</sup>. It is preferable to use the 18-gauge rather than a 21-gauge needle with the triangulation technique, as its stiffness provides better stability to help maintain angle of entry<sup>[39]</sup>.

#### **Comparison of Bull's eye and Triangulation technique**

In the Triangulation technique, the puncture is along the stone axis, *i.e.*, in alignment with the infundibulum. This decreases the need for excessive torque on the renal parenchyma by the rigid instruments, which may cause renal trauma and bleeding<sup>[20]</sup>. Tepeler *et al.*<sup>[10]</sup> did a comparison of the Bulls' eye and the Triangulation techniques and found no difference between the two as regards operation time, fluoroscopy screening time, duration of hospitalization and blood transfusion rate. They found a slightly greater drop in hematocrit and complication rate in the group undergoing access by the Bull's eye technique as compared to the Triangulation technique. However, the difference was not statistically different.

The advantage of the triangulation technique over the eye-of-the-needle" technique is that the needle cannot be passed too deeply because the depth of advancement is monitored continuously<sup>[39]</sup>. Also, the triangulation technique alone fulfills the five criteria of a successful puncture<sup>[41]</sup>. The disadvantage of the triangulation technique is that maintaining both the medial-lateral and cephalo-caudal planes are difficult

because both are not being monitored at the same time as in the "eye-of-the-needle" technique. Complex visual spatial skills are required in performing this task when using a C-arm fluoroscopy unit, especially by the novice surgeon<sup>[44]</sup>. It is at this juncture that multiple attempts are needed by the urologists and excessive use of fluoroscopy occurs especially by a beginner. This is also the aspect which has the steepest learning curve for an urologist getting trained in percutaneous nephrolithotomy<sup>[45]</sup>. Usually, during the learning curve the problem comes in the assessment of depth with the C arm in the oblique position. Whether the needle is superficial or deep to the calyx has to be ascertained by the surgeon and adjustments made accordingly<sup>[46]</sup>. The easiest way to determine this would be to place another needle on the skin surface over the target calyx. If the calyx is between the two needles than the puncture needle is deep and should be adjusted superficially. If the target calyx is below the two needles, then the puncture needle is superficial and should be adjusted towards the depth.

#### **Modifications of triangulation technique**

Several new approaches and refinements have been described to improve access and reduce the learning curve of the surgeon. Mues *et al.*<sup>[47]</sup> described a geometric model to create a plane of coincidence between the C arm and the needle, each at the same angle of 20°-30° from the targeted calyx, but in opposite directions. For lower pole access, the C arm is rotated cranially 30° from the vertical plane and a needle is advanced from a position distal to the calyx, rotated caudally 30° from the vertical plane. For mid pole and upper pole calyceal access the C arm is rotated 20° away from the surgeon and the needle is advanced from a position lateral to the calyx at an angle of 20° towards the surgeon from the vertical plane. During the procedure the C arm remains fixed and the needle is advanced till the point of coincidence between the calyx and the needle tip is reached. This technique avoids the need for C arm manipulation and thus potentially reduces time need to achieve the puncture<sup>[41]</sup>. This technique, however, requires a plumb, protractor and ruler to calculate, and confirm the necessary measurements. Also, it presumes that the angle of convergence would be 30° at the lower pole and 20° at the other poles. Considering the wide variations in the structure of the kidney that occurs with the varying degrees of hydronephrosis that occurs, this may not necessarily be always true. Liatsikos *et al.*<sup>[48]</sup> described a technique to take advantage of the ability of triangulation technique to target a calyx from a preselected puncture site. After clearing the calculus from the initial puncture site, the sheath is withdrawn and additional calices which need to be approached for complete stone clearance are punctured from the initial puncture site. A single nephrostomy tube can be left despite multiple entries through the single incision<sup>[42,48]</sup>. The tracts are from a single site but in



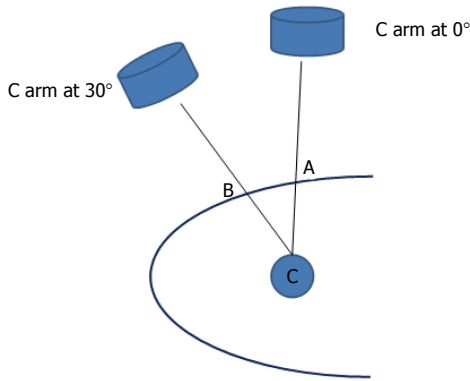
different directions. This does not reduce the chance of complications. Moreover, attempt of maneuvering the rigid nephroscope may increase the torque on the renal parenchyma with resultant increase in bleeding. Mozer *et al*<sup>[49]</sup> have described a computer generated system which can be used to project the ultrasound nephrostomy tract onto fluoroscopic images virtually. The surgeon, thus, has the benefit of having preview to a three dimensional anatomy while doing the procedure instead of the usual two dimensional fluoroscopy picture. Though exciting, it requires a special system which is not routinely available. Newer C arms utilizing softwares to provide a three dimensional picture have also been used to obtain renal access in animal models<sup>[50]</sup>. Robot assistance for fluoroscopic percutaneous renal access has also been studied and is under evaluation<sup>[51,52]</sup>. These futuristic techniques have not gained widespread acceptance. A study comparing robot assisted renal access with standard manual access showed that though the mean number of access attempts was comparable, the robot took lesser time to achieve puncture (10.4 min vs 15.1 min). However, conversion to manual access was needed in 3 cases where the robot was unsuccessful<sup>[53]</sup>. A stereotactic localization system with specially designed instruments have been described by Li *et al*<sup>[54]</sup>. It uses the Pythagoras principle of right angle triangle to calculate the depth of puncture. Then using specifically designed and patented instruments the puncture is made with precalculated depth and angle of puncture. The same instrument is then used for dilatation of the tract. The authors found that their technique is associated with higher efficiency, better stone clearance and lower morbidity, which they attribute to the greater accuracy of the puncture. This technique was not found to be useful when the puncture angle was less than 30°, because the buttocks of the operator would be in the way. Also the authors generally selected the puncture point with the same distance vertically and horizontally, which means at 45° from the skin to the stone. This again makes the principle of puncturing quite rigid as the variations in the pelvicalyceal anatomy may preclude adherence to such rigid principles. Recently, Hatipoglu *et al*<sup>[55]</sup> described a monoplanar access technique. The chosen calyx is marked with a clamp. For the lower pole puncture the needle is placed 1 cm below and medial to the 12<sup>th</sup> rib. The needle is placed with a 30° angle to the sagittal plane and is directed toward the desired calyx. If puncture fails, the needle is retracted approximately 1 cm intracorporeally, and its angle of entry adjusted on the same vertical plane and reinserted. For the mid and upper pole puncture the needle is held perpendicular to the spinal column and at a nearly 30° to the horizontal plane to access targeted middle and upper calyces to reach the pelvis and lower poles. The authors proposed that as the C arm is fixed in an anteroposterior position and thus rotation is avoided, the operative time needed for puncture is less<sup>[55]</sup>. However, there were no upper pole punctures in this study. In the initial learning curve

a surgeon would find this technique difficult to master. Also, using a pre fixed puncture point on the skin for all lower pole punctures and directing the needle at a fixed angle of 30° to the sagittal plane may not always be a correct approach especially considering the variation in the position and direction of the lower pole calyces.

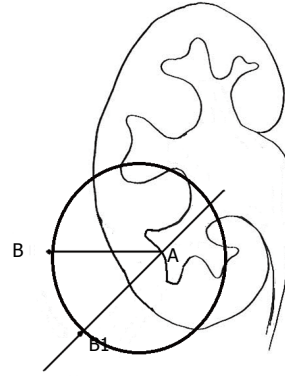
### Hybrid technique

Rationale thinking suggests that the three most important things needed to achieve a successful percutaneous renal puncture are the site of skin entry, the angle of entry and the depth at which the puncture is achieved. Determining the correct point of skin puncture is important in the triangulation technique because a skin puncture that is too medial or lateral to the desired optimum point of entry would result in a tract of variable length and angle of entry in the calyx. This would interfere with proper access and would cause excessive torque on the parenchyma during maneuvering of the rigid nephroscope in the pelvicalyceal system. The literature does provide guidelines when it comes to determining the site of skin puncture. To avoid injury to the colon, the puncture should be medial to the posterior axillary line but not too medial as it would traverse the paraspinal muscle causing increased postoperative pain and would probably be directly on the renal pelvis without traversing the renal parenchyma. The puncture that is too close to the rib may injure the intercostal nerve and vessels and hence is to be avoided. For lower-pole access, the skin puncture should be 1 cm inferior and 1 cm medial to the tip of the 12<sup>th</sup> rib<sup>[20,55-57]</sup>.

We have described the technique of determining the site of skin puncture, which amalgamates the advantages of both the bull's eye and triangulation technique and hence is called as the hybrid technique<sup>[41,57]</sup>. With the C arm at 0°, the site of skin corresponding to the target calyx is marked as point A. The C arm is then rotated 30° towards the surgeon. The point on the skin corresponding to the target calyx and forming a bull's eye with the needle is marked as point B. In The Bull's eye technique we take a puncture at the point B. However in the Triangulation technique, the puncture is along the stone axis in alignment with the infundibulum. If we take the target calyx as the center of a sphere, then we have an imaginary circle on the skin where the point A is the center of the circle. The distance from point A to B will be the radius of the circle. The radius remains the same irrespective of the direction in which it is measured from the center of the circle. Thus, when we take a line along the stone axis where we intend to take a puncture—the site of skin puncture is marked using this principle. This means that the point B1 is marked on the skin such that the distance from point A to B1 is equal to the distance between A to B, *i.e.*, the radius of a circle with the target calyx being its center. This is how we determine site of skin puncture in triangulation technique<sup>[57]</sup>



**Figure 7 Hybrid technique.** Point C is the calyx to be punctured. Point A corresponds to the Point C with the C arm at 0°. Point B corresponds to the point C with the C arm rotated towards the surgeon by 30°. The needle held at Point A or B is seen as a Bull's eye effect on the C arm monitor. The distance between points A and B is measured.



**Figure 8 Hybrid technique.** "A" is the point on the skin which corresponds to the targeted calyx with the C arm at 0 degree and is the center of an imaginary circle. The distance between "A" to "B1" is equal to the distance between the "A" to "B", i.e., the radius of the circle.

(Figures 7 and 8).

Once the site of puncture is determined the next critical step is to access the centre of a posterior calyx with the needle directed at an appropriate angle. This step of hitting the calyx at the depth often requires maneuvering the C arm in different directions, either towards the surgeon (in bull's eye technique) or in an oblique cephalo-caudal direction (for the triangulation technique) and requires understanding a three dimensional anatomy on a two dimensional fluoroscopy monitor. This step requires maintenance of the needle in one plane while making the adjustments in the other plane and not surprisingly, multiple attempts are needed and excessive use of fluoroscopy occurs, at this step, especially by a beginner<sup>[20,45]</sup>. Maintenance of needle orientation in one plane while making the adjustment in the other plane is critical for a proper puncture. This is also the aspect which has the steepest learning curve for an urologist getting trained in percutaneous nephrolithotomy<sup>[45]</sup>.

We describe our technique of using a simple mathematical principle to determine the angle and depth of puncture in fluoroscopy guided percutaneous renal access in prone position. We have used it in > 150 cases for lower, mid and upper pole punctures with > 95% success in first attempt and no pleural, visceral or hemorrhagic complications. This has recently been accepted for publication.

In the Bull's eye technique, the angle at which the needle is seen as a dot is the angle at which the puncture is made. Our Hybrid technique utilizes this principle. With the needle at point B and the C arm rotated 30° towards the surgeon and the needle forming the Bull's eye; the angle that the needle makes with the skin surface is measured using a protractor (Figure 9). One needs to take care that the protractor is held parallel to the operating table. Using the principle of sphere and circle as described earlier; if we are hitting the calyx by using the triangulation technique from the point B1-the angle of puncture

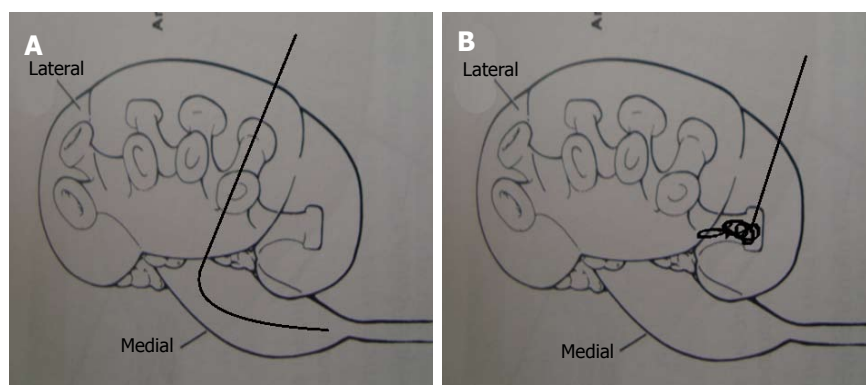


**Figure 9 Hybrid technique.** The angle which the needle makes with the skin surface is measured using a protractor.

would be the same with probably variations of 1-2 degrees due to the not so perfectly flat contours of the body surface. The third component of the hybrid technique is to determine the depth of puncture. What we have till now is an imaginary triangle (Figure 9) where we know: (1) One side - the distance between point A to B which is marked on the skin; (2) One angle - which is 90° with the C arm at 0°; and (3) Another angle- which is measured using the protractor at the point B.

With this information; by using the Universal triangle solver application from Google play store we can determine the depth. In this application, if we put the two angles and one side then, by the law of sines, it calculates the other two sides and the angle. For example, if the distance AB is 4 cm and the angle calculated by the protractor is 65° and with the other angle always being 90°- by universal triangle solver- the depth will be 9.5 cm.

The same principle can be applied in the triangulation technique. The C arm is brought to 0°. The line of puncture is determined in alignment with infundibulum from point A. On this line the point B1 is marked. From the Point B1 access can be obtained



**Figure 10 Calyceal puncture.** A: If the posterior calyx is punctured than the glide wire passes easily in the pelvis; B: if the anterior calyx is punctured than the glide wire gets coiled in the calyx before it makes its way to the pelvis.

using the triangulation technique (Figure 8). The angle of puncture is as determined by the protractor earlier using the bull's eye principle. The depth of puncture is the same as calculated earlier. As the angle of entry is known and the depth pre calculated, the needle is advanced with the C arm 0° position only (without the need to take it in oblique position) and the puncture is made.

In the technique described by us we have assumed the target calyx as the centre of a sphere. If we have to hit the centre of a sphere from the surface, the distance traversed from any point on the surface to the centre would be the same. Hence once we have marked the point B on the skin surface using the bull's eye technique and then mark point B1 for the skin entry using the triangulation technique; then the distance from C to B or from C to B1 will be the same, *i.e.*, the radius of the sphere. Also the angle of entry from B or from B1 towards point C would be nearly the same, with only minor difference, because of the not so perfectly flat contours of the body.

But, this minimal difference would not cause any major hindrance in achieving access by the technique described because the angle of puncture and thus the trajectory of the needle would not have much variation. This was seen by us in our study. The difference between the calculated and the actual depth ranged from 0-3 mm. Also, as the angle of entry is known the fluoroscopy screening time and the time needed to achieve puncture decreases as multiple movements of the C arm are not required.

The technique described by us is applicable for both the bull's eye and the triangulation method. It describes the three most important things needed to achieve a successful percutaneous renal puncture: the site of skin entry, the angle of entry and the depth at which the puncture is achieved. It relies on simple tools. There could be some errors which could creep in especially if the protractor is not held parallel to the operating table, but this could be overcome easily with minimal experience (and the assistant telling that the protractor is parallel to the table or not). But

so far this technique has not been compared with other techniques. The applicability and validation of this technique in the hands of others is yet to be ascertained. This would need a controlled prospective study involving many surgeons of equal experience and comparison with the traditional technique. It would then ascertain whether this technique is associated with a lesser fluoroscopy time, more accuracy and lesser learning curve as proposed by us. The grade of hydronephrosis can affect the puncture with the access being relatively easier for higher grades of hydronephrosis. However, if the angle, *i.e.*, the trajectory of puncture is correct, as described by this technique, the puncture would be easier and precise even in lesser grades of hydronephrosis.

## CONFIRMATION OF PUNCTURE OF POSTERIOR CALYX

If air has been instilled during opacification of the pelvi calyceal system, air will be aspirated followed by a free flow saline especially if it is instilled through the ureteric catheter. After this when the glide wire is passed, while maintaining the angle of the needle, it enters the pelvis easily. No manipulation is needed. On the contrary if the anterior calyx has been punctured than the glide wire will be coiled in the calyx, will not enter the pelvis easily or will do so only after much manipulation (Figure 10).

## PASSAGE OF GLIDE/GUIDE WIRE

If a 21G needle has been used for puncture than a 0.018 inch guide wire is passed initially which has to be exchanged later for a 0.035 inch guide wire. If a 18 G needle has been used than 0.035 inch glide wire can be passed. Initially a J tip Teflon coated guide wire was used. Nowadays the use of an angled tip hydrophilic glide wire is increasing. The maneuverability, resistance to kinking and ease with which it can be negotiated in the ureter across an impacted calculus or be coiled in a distant calyx are the distant benefits of this wire.

But the slippery nature of the hydrophilic wire makes it prone to displacement. Hence, it should be replaced with a stiffer wire such as a 0.035 inch Zebra or an Amplatz super stiff guide wire<sup>[21,41]</sup>. Another caution which needs to be exercised in passing the hydrophilic glide wire is to keep it wet. The dried tip can be stiff and cause inadvertent perforation of the collecting system.

## INCISING SKIN AND FASCIA

The skin should be incised adequately so that the dilators and desired size of Amplatz sheath can be introduced easily. The correct way of incising the fascia would be to use the knife along the needle under fluoroscopic guidance as a lumbotome. The fascia should be incised in two planes at right angles to each other. This is especially important in patients who have scarring as a result of previous surgery. One may use an 18 G coaxial fascial incising needle (Cook) taking care to avoid lacerating the nearby sub costal or intercostal neurovascular bundle on the inferior rib margin<sup>[21]</sup>.

## GUIDE WIRE AND SAFETY WIRE

Traditional teaching gave much emphasis on the placement of a safety guide wire to access the tract in the event of the inadvertent slipping out of the working guide wire<sup>[20]</sup>. Many surgeons nowadays do not find it necessary to place a second safety wire especially if the guide wire is passed all the way in the bladder and more so it is a super stiff guide wire<sup>[21]</sup>. However during the learning curve it is prudent to have a safety wire. This can be introduced alongside the initial wire using a dual lumen catheter or 8/10Fr coaxial dilator of the dilatation canula (Karl Storz)<sup>[20,21,41]</sup>. Dilatation should be done over the stiff wire and not over the slippery hydrophilic wire.

## TRACK DILATATION

The dilatation of the tract for creation of the nephrostomy access is an integral step of PCNL. Tract dilatation is performed to increase the size of the percutaneous wire access so that working instruments can be inserted in the pelvi-calyceal system (PCS). The size of the tract should be increased to 24 or 30 Fr size in most cases with the use of specialized dilators. The function of the dilator is to enlarge the tract in a noninvasive manner and to make renal access easier. The dilated tract is then maintained by placement of an Amplatz sheath.

Tract dilatation can be acute or chronic<sup>[58]</sup>. Chronic dilatation is done by placement of a percutaneous nephrostomy tube, which is gradually dilated over few days by sequentially replacing it by larger tubes. Acute dilatation is done just before the therapeutic

procedure. The tract is dilated either by sequential (Amplatz dilators) or telescopic coaxial dilators (Alken dilators). These are rigid dilators. Balloon dilators are also in vogue and have results similar to rigid dilators. The chronic dilatation approach was once the conventional method by which renal access surgery such as PCNL were done; however, in recent years one stage acute dilatation method has become preferred due to its low risks of patient morbidity and decreased time, which allows less room for complications.

### *Alkens dilators*

These are rigid metal dilators that are introduced over a central guide rod. Progressively enlarging coaxial stainless steel dilators help to dilate the tract from the 8 Fr guide rod up to 30 Fr. The guide rod has a round bulbous end prevents the sequential dilators from over-shooting. The advantages of the Alkens dilator system are that it is reusable, hence inexpensive and importantly is able to dilate even when there is dense perinephric scarring<sup>[59]</sup>. The disadvantage is that the same characteristics that make the Alkens dilator so effective are also the reasons why the rigid metal dilators can do considerable damage.

### *Amplatz dilators*

These are semi rigid plastic dilators that are passed over an 8-Fr PTFE guiding catheter that fits over a 0.035-inch guide wire. They can also be passed over a guide rod. The dilators are passed one after the other, not coaxially like the rigid metal dilators but progressively, by advancing one dilator, removing it, advancing the next larger dilator, and so on until the final tract diameter is achieved. Finally, the working sheath is passed over the final dilator and then the dilator and 8-Fr catheter are removed, leaving the working wire and sheath in place. The dilators are made in increments of 2 Fr, but if the tissue being dilated is soft, then not every dilator needs to be used.

The advantages of Amplatz dilators are that trauma experienced by the collecting system is theoretically less probable than the trauma experienced by the collecting system using rigid metal dilators, but the disadvantage is that bleeding can happen each time a dilator is withdrawn. As these are disposable dilators, they are more expensive than the Alkens dilators.

There have been many comparative studies<sup>[59-63]</sup> between the two dilator systems but experienced urologists have found no difference between the two systems in terms of safety. Alkens dilators may be preferred in patients who have tight fitting staghorn calculi, as Amplatz dilators need some space in the calyx for dilatation. In calyces that have no space, the dilatation may remain short due to tapered end of the dilator.

### *Balloon dilators*

Here a pressure balloon is used for rapid tract



making<sup>[64]</sup>. The Amplatz sheath is back loaded on the balloon and is placed once the balloon is adequately distended. The balloon dilator is expensive and may be difficult to use in patients with densely scarred tissue. The dilators may have an advantage when operating on a hyper mobile kidney. As it is a single step dilatation that causes tamponade, the bleeding is expected to be less, but not all studies have documented less bleeding and transfusion as compared to the Alkens and Amplatz dilators<sup>[59,65-67]</sup>.

In an effort to make tract making rapid, easy, and blood less, multiple single step techniques have been described. The simplest is using the largest Amplatz dilators without the initial smaller dilators. In difficult situations where scar tissue is present around the kidney, collings knife or plasma vaporization has been used for tract making<sup>[68]</sup>. Two new dilatation systems described have been a radially expanding single step dilator system<sup>[69]</sup> and the Spang system<sup>[70]</sup>. Both the systems of the advantage of not removing the needle and hence the dilatation is over a rigid system resulting in less chances of kinking of guide wire. Also the dilatation would be faster. However, so far it has been a single center experience and multi center experience with these two systems has not been described.

The important principles of tract dilatation are: (1) A proper planning of the procedure and correct choice of the calyx of entry is vital for the success of PCNL. This needs a study the radiologic images prior to the procedure; (2) The tract should be dilated only till the minor calyx. If over-dilatation happens, it can traumatize the infundibulum, renal pelvis or uretero-pelvic junction. Trauma to the anterior wall of the PCS can cause significant bleeding that may be difficult to control. It is always better to under dilate than to over dilate and cause trauma; (3) The success of tract making is dependent on maintaining the angle, depth, and the direction of the dilatation; (4) Every step of dilatation should be monitored on fluoroscopy; (5) The collecting system should be kept distended during dilatation by instilling in the system either contrast or saline. This is instilled by the OR assistant through the ureteric catheter; and (6) Adequate lumbotomy is important for safe dilatation.

## AMPLATZ SHEATH

The use of an Amplatz Sheath during percutaneous renal procedures has become standard. No matter the type of dilator used, rigid or balloon, or the technique of track dilation, one-step or multi-stepped, an Amplatz sheath is always used. The Amplatz sheath serves many purposes: (1) Amplatz sheath maintains the tract during procedure; (2) It causes tamponade of the tract and reduces bleeding. The beveled end of the Amplatz sheath can be used to tamponade a part of renal parenchyma that is actively bleeding<sup>[71]</sup>; (3) It protects the renal parenchyma from injury by the

instruments used in renal procedures; (4) The use of Amplatz sheath maintains a low-pressure system and reduces fluid intravasation. Maintaining a low-pressure system would be important in patients with infected calculi as the risk of sepsis would reduce; and (5) Amplatz sheath helps in removal of calculi and prevents parenchymal injury by broken ragged stone edges.

## WHEN TO DO MULTIPLE PUNCTURES?

The amount of bleeding, parenchymal damage, morbidity as well as the risk to the patient increases with increase in the number of punctures<sup>[72]</sup>. It is important to plan the first puncture in a way that multiple punctures are avoided. Use of flexible nephroscopy and flexible ureteroscopy would also reduce the need for multiple punctures<sup>[73]</sup>.

Multiple accesses may be needed when treating large and complex stones and staghorn calculi. In this situation the first tract is made in a way that most stone bulk can be removed through it. The accessory tracts can be mini-PCNL tracts for peripheral small calculi. In this situation, upper calyx has an advantage as it affords a direct access to the upper calyx, renal pelvis, all components of the lower calyx and the upper ureter<sup>[74]</sup>. In selected situations where the calculi are smaller than the neck of the calyx, percutaneous calyceal lavage can be done to flush the calculi in the renal pelvis so that they can be picked up through the primary tract. If necessary, multiple tracts can be safely made in experienced hands with the intent of complete stone clearance<sup>[75]</sup>.

In a complex situation, the plan of management would be as follows: Make the primary tract in a way to clear maximum stone bulk. If access to a flexible nephroscopy with holmium laser is available, use this to prevent additional tracts. If these facilities are not available, percutaneous calyceal lavage, mini-tracts or accessory tracts can be made.

## HOW TO MAKE A SAFE SECOND TRACT?

The second or multiple tracts tend to bleed more than the primary tract because when the second tract is made, it is not possible to opacify the system. The puncture is more often directed to the stone and not to the calyx. Also the bleeding and fluid extravasation through the first tract can alter the anatomy. To prevent this, if a second tract is anticipated, it is better to place the guide wires in the calyces where the second tract is expected before the first tract is dilated. The advantage of a pre-placed guide wire is that the proper placement of the tract is possible but the disadvantage is that in some patients this wire may not need to be dilated. The advantages of a proper placed tract far outweigh the risk of a tract made

aiming for the stones.

## HOW TO MINIMIZE RADIATION?

The risk of radiation is quite high in patients with stone disease during their evaluation and treatment. Recently, two centers have studied the radiation dose in a patient with a primary acute stone event over a 1 year period. They found average radiation to which these patients were exposed was 29.7 mSv, and 20% of the patients were exposed to > 50 mSv<sup>[76]</sup>. This dose exceeds the International Commission on Radiological Protection recommendation on limits for occupational exposure to radiation, which is 20 mSv averaged over a 5-year period with not more than > 50mSv in any single year. In comparison, a typical CT of the abdomen and pelvis without contrast exposes patients to a median of 15 mSv<sup>[77]</sup>. Fluoroscopy during percutaneous nephrolithotomy is associated with radiation exposure not only to the patient but also to the surgeon and the operation theatre staff<sup>[78]</sup>. High Body Mass Index, high stone burden, and increasing number of access tracts are associated with an increased radiation exposure. Branched stones and the presence of hydronephrosis are associated with decreased radiation exposure<sup>[79,80]</sup>. Proper planning of the procedure by an experienced surgeon is very important for reduction of radiation exposure<sup>[81]</sup>. It is the duty of the surgeon to reduce this health hazard for all concerned. Following steps can be taken to minimize radiation during PNL: (1) The surgeon and the staff must always wear radiation protection gowns, thyroid guards and radiation protection gloves<sup>[82]</sup>; (2) It is important to limit the time of exposure to minimum necessary. Using short bursts of fluoroscopy and using the "last image hold" feature of the fluoroscopy unit reduces radiation exposure<sup>[83]</sup>; (3) The image intensifier should be placed as close to the patient as possible, fluoroscopy beam should come from under the table, be focused on the area of interest and a pulsed fluoroscopy mode should be used<sup>[25]</sup>. The use of air instead of iodinated contrast may further reduce the radiation exposure<sup>[84]</sup>; (4) Keeping the fluoroscopy unit foot pedal with the surgeon and thinking during and after screening are other small precautions which can decrease the radiation; (5) For lower pole punctures using triangulation technique tilt the C arm cephalad and *vice versa* for upper pole puncture; and (6) Hold the needle in a way that the hands get minimal radiation exposure. Use of an instrument to achieve this would reduce radiation exposure. Needles and dilators that have distance marked on them can help in reducing radiation as the fluoroscopy can be used once the needle-tip is near the kidney<sup>[40,85]</sup>.

## PREVENTING VISCERAL INJURY DURING PCNL

Any abdominal organ close to the kidney can be

injured during percutaneous renal surgery including the colon, duodenum, jejunum, spleen, liver, and biliary system. Such injury is always an accident and an effort is needed in preventing them. If it happens, early identification and treatment is very vital.

### Colonic injury

Colonic injury happens in about 1% percutaneous renal procedures in prone position. It is thought to be due to retro-renal position of the colon. It is more common on the left side when a lower calyx access is attempted<sup>[86]</sup>. Thin patients, elderly age group, dilated colon, prior colon surgery or disease, and the presence of a horseshoe kidney are additional risk factors<sup>[40,87]</sup>. It can also happen in patients who undergo significant weight loss in a short time like patients after bariatric surgery, ileal diseases and resections. A recent hypothesis proposed retro-renal colon to be an acquired anomaly<sup>[88]</sup>. Five patients developed colonic injury in the 2<sup>nd</sup> stage PCNL. All these patients had a long-standing large hydronephrosis that was initially drained by either a nephrostomy or a DJ stent. They proposed that the colonic mesocolon lengthens over the gradually dilating obstructed kidney. Once the kidney is de-obstructed, the kidney reduces in size but the long mesocolon persists. The colon with the long mesocolon drops posterior to kidney forming a retro renal colon.

Prevention of colonic injury is very difficult. In patients who are predisposed to colonic injury, a pre-operative CT scan in prone position could help identify the position of colon in relation to the proposed tract. Awareness of the colonic gas bubble on fluoroscopy at the time of making access and monitoring any changes in the bubble could help prevent this injury. It would be possible to identify the overlying colon if a sonography guided puncture is attempted.

### Liver and splenic injury

Injury to normal sized liver and spleen are very rare during PCNL and are likely to occur if the puncture is above the 10<sup>th</sup> rib<sup>[89,90]</sup>. In patients with significant splenomegaly and hepatomegaly pre-operative CT scan could be used to decide a safe access. In rare situations, CT guided access could be made. Pre-operative awareness and planning is the only way to prevent these injuries.

### Pleural injury

Pleural injury is a definite risk associated with supra-costal access. All supra costal tracts traverse the diaphragm and hence there is risk of damage to the pleura and lung. The surgeon should be aware of this risk while undertaking Supracostal puncture<sup>[91]</sup>. The risk increases as the tract moves higher on the intercostal space. The risk that is about 4% in supra 12<sup>th</sup> rib access increases to nearly 20% in supra 11 rib access<sup>[92]</sup>.

To understand pleural injury during PCNL, it is

important to understand the pleural anatomy. The parietal pleura crosses the 12<sup>th</sup> rib such that the medial half is covered by the pleura while the lateral half of the rib is not covered by the pleura. In the mid scapular line, while the parietal pleura is at the level of the 12<sup>th</sup> rib and the visceral pleura is at the level of the 10<sup>th</sup> rib,. The parietal and visceral pleura ascend cranially and laterally on the ribs, and further rise in deep expiration<sup>[91,93]</sup>.

To prevent pleural violation<sup>[91]</sup>: (1) Make the Tract lateral to the mid scapular line; (2) As far as feasible, stay below the 10<sup>th</sup> rib; (3) Tract making should be performed in deep expiration; and (4) Tract should be kept to the minimum necessary size.

Based on the above mentioned anatomical caveats it would be rational to suggest that tracts below the 11th rib made lateral to the mid scapular line would miss not only the visceral pleura but mal also miss the parietal pleura. Tracts made through the parietal pleura may not be of clinical significance. Use of an Amplatz sheath would further mitigate major complications by preventing leakage of the irrigation fluid in the pleural space.

An anesthetist who understands the procedure and is involved during the procedure is important, as he would maintain the patient in deep expiration when the tract is being made. If the individual case demands higher tract, there is no harm in making it. Use of thoracoscopy control would make this safer<sup>[94]</sup>. The pleural fluid collection, if occurs, can be easily managed by placing a chest drain at end of the procedure. It is vital to check the costo-phrenic angle at the end of the supra-costal access. A clear costo phrenic angle on fluoroscopy at the end of the procedure is a proof that pleura have not been violated<sup>[95]</sup>.

## PROBLEMS DURING ACCESS

### **Failure to opacify the system**

**Cause:** This uncommon occurrence can occur either due to the ureteric catheter slipping out or a tightly impacted calculus preventing passage of contrast across it. **Prevention:** The ureteric catheter needs to be fixed to the per urethral catheter inserted initially so that it does not slip out while making the patient prone. Using a hydrophilic glide wire also helps in negotiating the wire and then the ureteric catheter across the calculus.

**Remedy:** For a tightly impacted calculus, where the contrast does not go across it. Keeping the patient in "head-low" position or reducing the concentration of the contrast (increase the dilution) may help some contrast go beyond the blocking calculus. If this does not work, then either an ultrasound guided puncture can be attempted or one can use a Chiba needle to opacify the system. Chiba needle is a much finer needle as compared to the initial puncture needle hence is likely to be less traumatic<sup>[96]</sup>. The needle

is introduced around 2 cm lateral to the vertebral transverse process at L1-2 level. At this site it is likely to hit the renal pelvis. The CT scan images could help in identification of exact site of renal pelvis. Once the PCS is entered with a Chiba needle (confirmed by aspiration of urine from PCS), opacify the system and make the standard tract through the chosen calyx.

### **Extravasation of contrast**

**Cause:** Extravasation of the contrast is an unfortunate problem. It is important to avoid this situation, as extravasation would happen before the main procedure begins, and would complicate the further access making. The extravasated contrast would make the tract making difficult and also hamper the radiologic confirmation of stone clearance post procedure. The most common cause is when an enthusiastic assistant instills a large volume of contrast under high pressure. Rarely, the contrast may extravasate from an improperly placed ureteric catheter. This would be more common in patients with large impacted ureteric calculi with infection. It may also happen intra-op when the first attempt at needle insertion is not satisfactory and the contrast leaks from the needle puncture site that is made in the collecting system.

**Prevention:** To prevent extravasation of contrast, inject diluted contrast slowly while keeping the ureteric catheter in the pelvis so that sudden distension of the system with consequent extravasation does not occur. It is extremely important to instruct the assistant to instill a small amount of contrast gradually at a very low pressure. The volume of the normal collecting system is 5-8 mL; hence gradual instillation of small volume is vital.

**Remedy:** The problem can be salvaged in multiple ways: (1) Give diuretic and wait for the contrast to get absorbed. The concentration of the extravasated contrast would significantly reduce if you wait for about 15 minutes after a frusemide injection; (2) Use concentrated contrast that would help in identification of the PCS through the dilute extravasated contrast. The tract needs to be made fast before the concentrated contrast extravasates and compounds the problem; (3) Use of air-pyelogram to identify the PCS. The similar problem of air extravasation can happen through the needle hole in the cortex; (4) Ultrasound guided percutaneous access is a good option. However, even this technique would be difficult after contrast extravasates. Do not attempt air pyelogram, if you want to do an ultrasonography; (5) Very rarely, it may be desirable to stage the procedure and re-attempt access after 48 h; (6) Grasso *et al*<sup>[97]</sup> initially described ureteroscopically assisted percutaneous renal access as a salvage procedure in difficult cases. This can be utilized in cases where significant extravasation has occurred. The major hindrance is the availability of a flexible scope, which is not the case in many

developing countries; and (7) Giannakopoulos *et al.*<sup>[98]</sup> have described the use of an angiographic catheter to salvage such situation. A 0.038-inch guide wire is passed through open-end ureteral catheter which is then removed and an angled-tip angiographic catheter is passed. The radiopaque tip of the angiographic catheter is easily seen on the fluoroscopy despite significant extravasation of contrast. A guide wire is then passed through the angiographic catheter. It is manipulated and brought in a calyx which is to be punctured. The angiographic catheter is then brought till the calyx and the puncture is made aiming at the tip of the catheter. The intravenous urogram film or an initial normal fluoroscopic image before extravasation, which is captured on the second monitor of the fluoroscopy unit is very helpful in manipulating the guide wire and catheter in the proper calyx. The correct position of the catheter in a posterior calyx can also be confirmed by rotating the C-arm<sup>[98]</sup>.

#### **Inability to puncture**

**Cause:** Inability to puncture is often a technical problem. This happens either due to the inexperience of the operator or due to technical difficulty commonly while attempting puncture of a non-dilated system. This could be related to incorrect choice of the calyx for puncture.

**Prevention:** In the initial learning phase, presence of a more experienced colleague goes a long way in minimizing the learning curve and overcoming difficulties during the procedure. It is important to keep the PCS adequately filled for ease of puncture. Ask the assistant to continuously flush fluid in the ureteric catheter so that the system remains distended. If despite multiple attempts it is still difficult, reassess the pre-operative radiological studies and re-plan the puncture. Add a drop of methylene blue or betadine solution to the contrast; aspiration of the colored fluid (blue or brown) from kidney would give confidence of correct puncture<sup>[99]</sup>.

**Remedy:** In a non-dilated system, fluoroscopy guided puncture is usually feasible using the techniques described above. If the surgeon is worried about trauma to the kidney, then it is prudent to use a 21 G needle for initial puncture and pass a 0.018 inch guide wire, which can be exchanged for a 0.035 inch stiffer guide wire<sup>[40]</sup>. If these attempts fail, take the help of a senior colleague from the department. An interventional radiologist may help with difficult punctures. Re-planning the procedure under CT scan guidance or ultrasonography guidance may rarely be needed<sup>[100]</sup>. Puncture would also be difficult in a patient who has a very thin renal cortex. The renal cortex tends to move away from the needle or to get tented by the needle rather than getting punctured. Forcefully pushing the needle in, once near the cortex, can help the needle enter the PCS. Forceful insertion would be

safe as in this patient with thin renal cortex the PCS is likely to be hugely dilated. Proper care of the wire once inserted is very important in these patients. It is also important to keep a safety wire if possible. If there were a tract loss, it would be very difficult to get inside these deflated hydronephrotic sacs. Puncture will also be awkward in very thin patients. As there is no perirenal fat pad, the kidney tends to get pushed by the needle. A bolster kept below the kidney can hold the kidney in place so that puncture can be made.

#### **Blood at tip of needle and not urine**

**Cause:** It is not uncommon to have made a puncture and after removing the trocar and aspiration have blood and not urine. This happens if the needle is in a blood vessel or needle is in renal parenchyma instead of in the pelvicalyceal system. It can also occur if multiple attempts have been made to achieve access.

**Prevention:** Avoid trauma by making multiple attempts of puncture using a 18 G needle, instead use a 21 G needle. Follow a proper puncture technique so that we aim at the calyx through the fornix.

**Remedy:** If after injecting saline through the ureteric catheter, the efflux clears then it suggests that the needle tip is in the collecting system and glide wire can be passed in the collecting system. If the efflux or the aspirated fluid is frank blood then the needle's position should be readjusted. This entails with drawing the needle and adjusting the medio-lateral and anteroposterior position. It is usually the depth of the needle which needs to be adjusted. Either the needle is superficial or deep to the desired calyx. This adjustment is best made after withdrawing the needle outside the parenchyma. Manipulations within the parenchyma cause trauma and should be avoided. The 3 finger technique described by Shergill *et al.*<sup>[46]</sup> is an attempt to help the junior trainee to overcome this difficulty. The tip of the needle should be towards the desired calyx with the C arm in the anteroposterior position or tilted towards or away from the surgeon or tilted in cephalo caudal direction. The surgeon should remember the medio-lateral adjustments should be made with the C arm at 0° and the depth adjustment should be made after tilting the C arm towards or away from the surgeon, as in Bull's eye technique or tilting it in the cephalo-caudal direction, as in the triangulation technique. An easy way to determine the depth would be to place another needle on the skin surface over the target calyx. If the calyx is between the two needles then the puncture needle is deep and should be adjusted superficially. If the target calyx is below the two needles, then the puncture needle is superficial and should be adjusted towards the depth. Use of the Hybrid Technique described above minimizes these problems.

#### **Inability to park the guide wire**

**Cause:** This occurs either if the glide wire is outside



the collecting system or if the calyx is completely occupied by a calculus. Rarely, inadvertent puncture of a renal cyst and aspiration of clear fluid can cause a mistaken assumption of a good puncture. But the glide wire does not enter the collecting system in such cases. If an anterior calyx is punctured then also the glide wire will not enter the pelvis easily (Figure 10B).

**Prevention:** Free flow of urine from the needle usually is a sure shot sign of a correct puncture. A hydrophilic glide wire usually passes easily in the pelvis even across an impacted calculus. As regards inadvertent cyst puncture, the ultrasound findings and the intravenous urogram or the CT scan picture should alert the surgeon regarding such a possibility.

**Remedy:** Re puncture or re-insert the glide wire if one suspects that the glide wire is outside the system. Injecting diluted contrast or diluted methylene blue can also confirm that the needle is properly positioned in the desired calyx. There may be a calculus blocking the passage of the wire down the ureter, in this situation, the second best place to park the wire would be a distant calyx. If the wire does not coil in distant calyx then keep as much length as possible of the wire in the punctured calyx<sup>[99]</sup>.

### **Kinking of guide wire**

**Cause:** This usually occurs due to forceful dilatation in the wrong direction and/or against resistance of the initial dilator. Once the guide rod is in place this problem cannot occur.

**Prevention:** The wire usually kinks at the level of the thoracolumbar fascia. Hence the fascia needs to be incised well before starting the dilatation. Use of super stiff wire is recommended due to its properties to resist kinking more than the PTFE guide wire<sup>[101]</sup>. The dilatation should be in the correct direction and with adequate force. A simple rule would be to achieve the 2/3 of the progress of the dilator by rotational screwing movements and 1/3 by force. If there is doubt regarding the correct direction then moving the glide wire gives a good indication. If the wire moves freely then it indicates that the direction and trajectory is correct. *Vice versa*, if the glide wire does not move freely then the direction and trajectory needs to be adjusted. This simple friction test can be of immense help in the initial learning of percutaneous renal access. Use of the 5 part PANG needle system largely avoids this problem<sup>[70]</sup>.

**Remedy:** If a kink has occurred then the initial dilator should be advanced close to the kink and it should be pulled inside the dilator. The correct direction should then be ascertained and further dilation should be done. At times a re-puncture is needed. If a safety wire has been inserted then it can be used for dilatation.

Recently Lezrek *et al*<sup>[102]</sup> have described a use of bi prong forceps to overcome renal mobility and prevent guide wire kinking during tract dilatation.

### **Under dilatation**

**Cause:** Under dilatation is a condition when the wire was initially well placed but during dilatation the dilators and the Amplatz sheath remained short of the PCS. This usually occurs early during the learning curve. Use of Amplatz dilators is also associated with this as the terminal taper end of the dilator enters the calyx but the amplatz sheath introduced over it does not enter the calyx and remains outside the collecting system. This may cause brisk bleeding as there is a portion of parenchyma that has been partially dilated, which does not have the tamponade effects of the Amplatz sheath. This situation needs rapid management.

**Prevention:** Flushing saline from the ureteric catheter during the process of dilatation helps in confirming that the dilator/ dilators are within the collecting system by seeing the efflux of saline from the dilators. Small frequent bursts of screening on the C arm confirm the correct position of the dilators.

**Remedy:** The treatment would depend on the position of the wire. If the wire is still inside the PCS, thread the guide rod on the wire so that the bulbous end of the rod is fluoroscopically positioned in the PCS. Using the flexible guide rod may be easy in this situation. Once the guide rod is placed, use Amplatz dilators to dilate the remaining non dilated tract and the reposition the Amplatz sheath in the collecting system. If the wire has also slipped out and the nephroscope is outside the parenchyma in the perirenal fat, it is important to find the hole in the renal capsule through which partial dilatation had been done. This can be identified as a site of bleeding in the parenchyma. To identify this site one may need to reduce the irrigation pressure of the nephroscope so that venous bleeding is visible. Once the site is identified, place the guide rod through the capsular hole and confirm its position on the fluoroscopy. A guide wire may be placed through the rod to make the access secure. Once this is done, the remaining dilatation could continue with Amplatz dilators. If no bleeding site is identified, for identifying the capsular hole some colored fluid will be needed. Methylene blue or betadine solution can be used. The methylene blue solution should be very dilute. Add just 1-2 drops of methylene blue in 10 cc normal saline. For betadine solution, undiluted betadine or betadine with one-in-one dilution could be used. Flush either solution through the ureteric catheter. Watch for egress of colored solution. Once identified, place a guide rod through that site and continue with the remaining dilatation. If despite colored solution, puncture site cannot be identified, attempts re-puncture and repeat

tract making. This may be difficult as the contrast may extravasate or the calyx may not fill due to leakage of contrast. Choosing an access through another calyx or sonography guided puncture may help. In a rare situation, it may be needed to stage the procedure. The puncture site seals in 48-72 h and a repeat procedure can be done after that time.

### Overdilatation

**Cause:** Over dilatation is a state when the dilators have traversed the opposite wall of the PCS and the Amplatz sheath is now placed anterior to the kidney. Forceful dilatation is the usual reason for this problem.

**Prevention:** Hold the guide rod firmly and dilate 2/3 by rotation and 1/3 by force. Attempt should be to dilate till the calyx and not till the calculus. It is better to under dilate than to over dilate.

**Remedy:** The problem with this complication is that when the Amplatz sheath is withdrawn back to get it in the PCS, the dilated anterior wall will not have any tamponade effect and is likely to bleed briskly. Also, the irrigation fluid would leak through the hole in anterior wall. Further Nephroscopy would become difficult as the PCS may collapse due to fluid leakage. The fluid that extravasates would cause significant fluid overload. Even the stone or stone fragments can migrate outside the PCS through the hole in the anterior wall. The Amplatz sheath needs to be withdrawn gradually till the sheath is back in PCS. The further plan after this would depend on the size of perforation and the amount of bleeding. If over dilatation has resulted in a small pelvic perforation than one can get back properly in the system and quickly finish the procedure without causing much extravasation. However if there is a large perforation or significant bleeding than it is prudent to insert a nephrostomy tube, abandon the procedure and live to fight another day. Always a keep a large bore nephrostomy tube during such situations. The second procedure can be staged after 3-4 d, as the perforation usually heals within this period<sup>[103]</sup>.

### Loss of tract

**Cause:** Loss of tract means initially that the tract was well made but during Nephroscopy or lithotripsy, the Amplatz sheath has slipped out of the PCS. This happens when the glide wire slips out before the dilatation is completed. At times during the fragmentation of the calculus, the guide wire comes out and if the amplatz is not held properly then it too can come out of the collecting system.

**Prevention:** To prevent loss of tract, the guide wire should be adequately parked in the collecting system or passed till the bladder. Use of a safety wire is another maneuver which can prevent a complete loss

of tract. Also using a super stiff guide wire instead of a slippery hydrophilic glide wire prevents complete loss of tract.

**Remedy:** The treatment depends on the position of the guide wire. If either the guide wire or the safety wire is well placed, it is possible to follow the wire endoscopically till the nephroscope is positioned in the PCS; the Amplatz sheath can be threaded over the nephroscope once the scope is well placed. If there is no wire, the steps are same as for under-dilatation. Look for the bleeding site or look for egress of the colored fluid. One important concern of the lost tract is that calculi or calculi fragments may extrude and can get misplaced in the perirenal fat. These calculi can be a source of persistent infection. If possible, an attempt should be made to remove all calculi. During endoscopic exploration of the perirenal space, reduce the irrigation pressure so that patient does not land in fluid overload. If despite care, the extruded calculi are misplaced in the perirenal fat, it is important to document that the radio-opaque shadows seen on post-op imaging are outside PCS. They would cause apprehension to the patient hence proper explanation to the patient is vital.

## CONCLUSION

"Good results of surgery are results of good surgery". This adage is most appropriate for a percutaneous renal access, for a correct access decides to a large extent the success of PCNL. More innovations would come in the management of renal calculus and technology would make percutaneous access easier in future; but adherence to the understanding of renal anatomy would still be the base on which a percutaneous renal access would be based.

## REFERENCES

- 1 **Rupel E**, Brown R. Nephroscopy with removal of stone following nephrostomy for obstructing calculus anuria. *J Urol* 1941; **46**: 177-182
- 2 **Goodwin WE**, Casey WC, Woolf W. Percutaneous trocar (needle) nephrostomy in hydronephrosis. *J Am Med Assoc* 1955; **157**: 891-894 [PMID: 13233046 DOI: 10.1001/jama.1955.02950280015005]
- 3 **Bissada NK**, Meacham KR, Redman JF. Nephrostoscopy with removal of renal pelvic calculi. *J Urol* 1974; **112**: 414-416 [PMID: 4414790]
- 4 **Karametchi A**, O'donnell WF. Percutaneous nephrolithotomy: an innovative extraction technique. *J Urol* 1977; **118**: 671 [PMID: 916071]
- 5 **Fernström I**, Johansson B. Percutaneous pyelolithotomy. A new extraction technique. *Scand J Urol Nephrol* 1976; **10**: 257-259 [PMID: 1006190]
- 6 **Morris DS**, Wei JT, Taub DA, Dunn RL, Wolf JS, Hollenbeck BK. Temporal trends in the use of percutaneous nephrolithotomy. *J Urol* 2006; **175**: 1731-1736 [PMID: 16600744 DOI: 10.1016/S0022-5347(05)00994-8]
- 7 **de la Rosette J**, Assimos D, Desai M, Gutierrez J, Lingeman J, Scarpa R, Tefekli A. The Clinical Research Office of the

- Endourological Society Percutaneous Nephrolithotomy Global Study: indications, complications, and outcomes in 5803 patients. *J Endourol* 2011; **25**: 11-17 [PMID: 21247286 DOI: 10.1089/end.2010.0424]
- 8 **Agarwal M**, Agrawal MS, Jaiswal A, Kumar D, Yadav H, Lavanja P. Safety and efficacy of ultrasonography as an adjunct to fluoroscopy for renal access in percutaneous nephrolithotomy (PCNL). *BJU Int* 2011; **108**: 1346-1349 [PMID: 21251187 DOI: 10.1111/j.1464-410X.2010.10002.x]
  - 9 **Ray AA**, Chung DG, Honey RJ. Percutaneous nephrolithotomy in the prone and prone-flexed positions: anatomic considerations. *J Endourol* 2009; **23**: 1607-1614 [PMID: 19630486 DOI: 10.1089/end.2009.0294]
  - 10 **Tepeler A**, Armağan A, Akman T, Polat EC, Ersöz C, Topaktaş R, Erdem MR, Onol SY. Impact of percutaneous renal access technique on outcomes of percutaneous nephrolithotomy. *J Endourol* 2012; **26**: 828-833 [PMID: 22283962 DOI: 10.1089/end.2011.0563]
  - 11 **Duty B**, Waingankar N, Okhunov Z, Ben Levi E, Smith A, Okeke Z. Anatomical variation between the prone, supine, and supine oblique positions on computed tomography: implications for percutaneous nephrolithotomy access. *Urology* 2012; **79**: 67-71 [PMID: 21820700]
  - 12 **Lashley DB**, Fuchs EF. Urologist-acquired renal access for percutaneous renal surgery. *Urology* 1998; **51**: 927-931 [PMID: 9609628 DOI: 10.1016/S0090-4295(98)00101-0]
  - 13 **Tomaszewski JJ**, Ortiz TD, Gayed BA, Smaldone MC, Jackman SV, Averch TD. Renal access by urologist or radiologist during percutaneous nephrolithotomy. *J Endourol* 2010; **24**: 1733-1737 [PMID: 20919919 DOI: 10.1089/end.2010.0191]
  - 14 **El-Assmy AM**, Shokeir AA, Mohsen T, El-Tabey N, El-Nahas AR, Shoma AM, Eraky I, El-Kenawy MR, El-Kappany HA. Renal access by urologist or radiologist for percutaneous nephrolithotomy - is it still an issue? *J Urol* 2007; **178**: 916-920; discussion 920 [PMID: 17632136 DOI: 10.1016/j.juro.2007.05.015]
  - 15 **Bird VG**, Fallon B, Winfield HN. Practice patterns in the treatment of large renal stones. *J Endourol* 2003; **17**: 355-363 [PMID: 12965059 DOI: 10.1089/089277903767923119]
  - 16 **Lee CL**, Anderson JK, Monga M. Residency training in percutaneous renal access: does it affect urological practice? *J Urol* 2004; **171**: 592-595 [PMID: 14713766 DOI: 10.1097/01.ju.0000104849.25168.6d]
  - 17 **Miller J**, Durack JC, Sorensen MD, Wang JH, Stoller ML. Renal calyceal anatomy characterization with 3-dimensional in vivo computerized tomography imaging. *J Urol* 2013; **189**: 562-567 [PMID: 23260557 DOI: 10.1016/j.juro.2012.09.040]
  - 18 **Edgcombe H**, Carter K, Yarrow S. Anaesthesia in the prone position. *Br J Anaesth* 2008; **100**: 165-183 [PMID: 18211991 DOI: 10.1093/bja/aem380]
  - 19 **Stevens WR**, Glazer PA, Kelley SD, Lietman TM, Bradford DS. Ophthalmic complications after spinal surgery. *Spine (Phila Pa 1976)* 1997; **22**: 1319-1324 [PMID: 9201834]
  - 20 **Miller NL**, Matlaga BR, Lingeman JE. Techniques for fluoroscopic percutaneous renal access. *J Urol* 2007; **178**: 15-23 [PMID: 17574053 DOI: 10.1016/j.juro.2007.03.014]
  - 21 **Ko R**, Soucy F, Denstedt JD, Razvi H. Percutaneous nephrolithotomy made easier: a practical guide, tips and tricks. *BJU Int* 2008; **101**: 535-539 [PMID: 17922862 DOI: 10.1111/j.1464-410X.2007.07259.x]
  - 22 **Sampaio FJ**. Renal anatomy. Endourologic considerations. *Urol Clin North Am* 2000; **27**: 585-607 [DOI: 10.1016/S0094-0143(05)70109-9]
  - 23 **Carriaco CW**, Zerlin JM. Sonographic measurement of renal length in children: does the position of the patient matter? *Pediatr Radiol* 1996; **26**: 553-555 [PMID: 8753670 DOI: 10.1007/BF01372240]
  - 24 **Sharma G**, Sharma A, Maheshwari P. Predictive value of decreased renal pelvis anteroposterior diameter in prone position for prenatally detected hydronephrosis. *J Urol* 2012; **187**: 1839-1843 [PMID: 22425050 DOI: 10.1016/j.juro.2011.12.093]
  - 25 **Park S**, Pearle MS. Imaging for percutaneous renal access and management of renal calculi. *Urol Clin North Am* 2006; **33**: 353-364 [PMID: 16829270 DOI: 10.1016/j.ucl.2006.03.003]
  - 26 **Thiruchelvam N**, Mostafid H, Ubhayakar G. Planning percutaneous nephrolithotomy using multidetector computed tomography urography, multiplanar reconstruction and three-dimensional reformatting. *BJU Int* 2005; **95**: 1280-1284 [PMID: 15892817 DOI: 10.1111/j.1464-410X.2005.05519.x]
  - 27 **Hopper KD**, Yakes WF. The posterior intercostal approach for percutaneous renal procedures: risk of puncturing the lung, spleen, and liver as determined by CT. *AJR Am J Roentgenol* 1990; **154**: 115-117 [PMID: 2104692 DOI: 10.2214/ajr.154.1.2104692]
  - 28 **Ghani KR**, Patel U, Anson K. Computed tomography for percutaneous renal access. *J Endourol* 2009; **23**: 1633-1639 [PMID: 19814578 DOI: 10.1089/end.2009.1529]
  - 29 **Bilen CY**, Koçak B, Kitiirci G, Danaci M, Sarikaya S. Simple trigonometry on computed tomography helps in planning renal access. *Urology* 2007; **70**: 242-245; discussion 245 [PMID: 17826479 DOI: 10.1016/j.urology.2007.03.079]
  - 30 **Sampaio FJ**, Zanier JF, Aragão AH, Favorito LA. Intrarenal access: 3-dimensional anatomical study. *J Urol* 1992; **148**: 1769-1773 [PMID: 1433604]
  - 31 **Schultheiss D**, Engel RM, Crosby RW, Lees GP, Truss MC, Jonas U. Max Brödel (1870-1941) and medical illustration in urology. *J Urol* 2000; **164**: 1137-1142 [PMID: 10992353 DOI: 10.1016/S0022-5347(05)67128-5]
  - 32 **Settlage J**. Derek Hodson: Teaching and Learning About Science: Language, Theories, Methods, History, Traditions and Value. *Science & Education* 2011; **20**: 393-396 [DOI: 10.1007/s11191-010-9266-7]
  - 33 **Kaye KW**, Reinke DB. Detailed caliceal anatomy for endourology. *J Urol* 1984; **132**: 1085-1088 [PMID: 6502793]
  - 34 **Sampaio FJ**, Mandarim-De-Lacerda CA, De Aragão AH. [The collector system of the kidney. Applied anatomy based on the analysis of 3-dimensional casts]. *J Urol (Paris)* 1987; **93**: 183-185 [PMID: 3680967]
  - 35 **Sampaio FJ**. Renal collecting system anatomy: its possible role in the effectiveness of renal stone treatment. *Curr Opin Urol* 2001; **11**: 359-366 [PMID: 11429494 DOI: 10.1097/00042307-200107000-00004]
  - 36 **Hwang TK**. Percutaneous nephroscopic surgery. *Korean J Urol* 2010; **51**: 298-307 [PMID: 20495691 DOI: 10.4111/kju.2010.51.5.298]
  - 37 **Eisner BH**, Cloyd J, Stoller ML. Lower-pole fluoroscopy-guided percutaneous renal access: which calyx is posterior? *J Endourol* 2009; **23**: 1621-1625 [PMID: 19814577 DOI: 10.1089/end.2009.1527]
  - 38 **Osman M**, Wendt-Nordahl G, Heger K, Michel MS, Alken P, Knoll T. Percutaneous nephrolithotomy with ultrasonography-guided renal access: experience from over 300 cases. *BJU Int* 2005; **96**: 875-878 [PMID: 16153221 DOI: 10.1111/j.1464-410X.2005.05749.x]
  - 39 **Abolhassani N**, Patel R, Moallem M. Needle insertion into soft tissue: a survey. *Med Eng Phys* 2007; **29**: 413-431 [PMID: 16938481 DOI: 10.1016/j.medengphys.2006.07.003]
  - 40 **Wolf JS**. Percutaneous approaches to the upper urinary tract collecting system. Campbell-Walsh Urology. 10th ed. Philadelphia, PA: Saunders Elsevier, 2011
  - 41 **Bernardo NO**. Percutaneous Renal Access Under Fluoroscopic Control. Smith's Textbook of Endourology. 3rd ed. Available from: URL: <http://onlinelibrary.wiley.com/book/10.1002/9781444345148>
  - 42 **Steinberg PL**, Semins MJ, Wason SE, Matlaga BR, Pais VM. Fluoroscopy-guided percutaneous renal access. *J Endourol* 2009; **23**: 1627-1631 [PMID: 19785549 DOI: 10.1089/end.2009.1528]
  - 43 **Bilen CY**, Aşçı R, Sarikaya S, Büyükalpelli R, Yılmaz AF. Laser-assisted fluoroscopic puncture: a new technique for accessing the kidney. *J Endourol* 2003; **17**: 485-491 [PMID: 14565879]
  - 44 **Ko R**, Razvi H. C-arm laser positioning device to facilitate percutaneous renal access. *Urology* 2007; **70**: 360-361 [PMID: 17826509 DOI: 10.1016/j.urology.2007.05.013]
  - 45 **Tanriverdi O**, Boylu U, Kendirci M, Kadihasanoglu M, Horasanli K, Miroglu C. The learning curve in the training of percutaneous nephrolithotomy. *Eur Urol* 2007; **52**: 206-211 [PMID: 17229522 DOI: 10.1016/j.eururo.2007.01.001]
  - 46 **Shergill IS**, Abdulmajed MI, Moussa SA, Rix GH. The 3-finger technique in establishing percutaneous renal access: a new and

- simple method for junior trainees. *J Surg Educ* 2012; **69**: 550-553 [PMID: 22677596 DOI: 10.1016/j.jsurg.2012.03.004]
- 47 **Mues E**, Gutiérrez J, Loske AM. Percutaneous renal access: a simplified approach. *J Endourol* 2007; **21**: 1271-1275 [PMID: 18042013]
  - 48 **Liatsikos EN**, Kapoor R, Lee B, Jabbour M, Barbalias G, Smith AD. "Angular percutaneous renal access". Multiple tracts through a single incision for staghorn calculous treatment in a single session. *Eur Urol* 2005; **48**: 832-837 [PMID: 16203078 DOI: 10.1016/j.eururo.2005.08.009]
  - 49 **Mozer P**, Conort P, Leroy A, Baumann M, Payan Y, Troccaz J, Chartier-Kastler E, Richard F. Aid to percutaneous renal access by virtual projection of the ultrasound puncture tract onto fluoroscopic images. *J Endourol* 2007; **21**: 460-465 [PMID: 17523896 DOI: 10.1089/end.2006.0168]
  - 50 **Soria F**, Delgado MI, Sánchez FM, Allona A, Jiménez Cruz JF, Morell E, Usón J. Effectiveness of three-dimensional fluoroscopy in percutaneous nephrostomy: an animal model study. *Urology* 2009; **73**: 649-652; discussion 652-654 [PMID: 19100601 DOI: 10.1016/j.urol.2008.09.063]
  - 51 **Cadeddu JA**, Bzostek A, Schreiner S, Barnes AC, Roberts WW, Anderson JH, Taylor RH, Kavoussi LR. A robotic system for percutaneous renal access. *J Urol* 1997; **158**: 1589-1593 [PMID: 9302179 DOI: 10.1016/S0022-5347(01)64285-X]
  - 52 **Bauer J**, Lee BR, Stoianovici D, Bishoff JT, Micali S, Micali F, Kavoussi LR. Remote percutaneous renal access using a new automated telesurgical robotic system. *Telemed J E Health* 2001; **7**: 341-346 [PMID: 11886670]
  - 53 **Su LM**, Stoianovici D, Jarrett TW, Patriciu A, Roberts WW, Cadeddu JA, Ramakumar S, Solomon SB, Kavoussi LR. Robotic percutaneous access to the kidney: comparison with standard manual access. *J Endourol* 2002; **16**: 471-475 [PMID: 12396439 DOI: 10.1089/089277902760367421]
  - 54 **Li X**, Liao S, Yu Y, Dai Q, Song B, Li L. Stereotactic localisation system: a modified puncture technique for percutaneous nephrolithotomy. *Urol Res* 2012; **40**: 395-401 [PMID: 22057205 DOI: 10.1007/s00240-011-0434-2]
  - 55 **Hatipoglu NK**, Bodakci MN, Penbegül N, Bozkurt Y, Sancaktar AA, Atar M, Söylemez H. Monoplanar access technique for percutaneous nephrolithotomy. *Urolithiasis* 2013; **41**: 257-263 [PMID: 23564416 DOI: 10.1007/s00240-013-0557-8]
  - 56 **Lang EK**. Percutaneous nephrostolithotomy and lithotripsy: a multi-institutional survey of complications. *Radiology* 1987; **162**: 25-30 [PMID: 3786771 DOI: 10.1148/radiology.162.1.3786771]
  - 57 **Sharma G**, Sharma A. Determining site of skin puncture for percutaneous renal access using fluoroscopy-guided triangulation technique. *J Endourol* 2009; **23**: 193-195 [PMID: 19220081 DOI: 10.1089/end.2008.0170]
  - 58 **Handa RK**, Matlaga BR, Connors BA, Ying J, Paterson RF, Kuo RL, Kim SC, Lingeman JE, Evan AP, Willis LR. Acute effects of percutaneous tract dilation on renal function and structure. *J Endourol* 2006; **20**: 1030-1040 [PMID: 17206897 DOI: 10.1089/end.2006.20.1030]
  - 59 **Davidoff R**, Bellman GC. Influence of technique of percutaneous tract creation on incidence of renal hemorrhage. *J Urol* 1997; **157**: 1229-1231 [PMID: 9120908 DOI: 10.1016/S0022-5347(01)64931-0]
  - 60 **Ozok HU**, Sagnak L, Senturk AB, Karakoyunlu N, Topaloglu H, Ersoy H. A comparison of metal telescopic dilators and Amplatz dilators for nephrostomy tract dilation in percutaneous nephrolithotomy. *J Endourol* 2012; **26**: 630-634 [PMID: 21999400 DOI: 10.1089/end.2011.0291]
  - 61 **Falahatkar S**, Neiroomand H, Akbarpour M, Emadi SA, Khaki N. One-shot versus metal telescopic dilation technique for tract creation in percutaneous nephrolithotomy: comparison of safety and efficacy. *J Endourol* 2009; **23**: 615-618 [PMID: 19335153 DOI: 10.1089/end.2008.0330]
  - 62 **Li Y**, Yang L, Xu P, Shen P, Qian S, Wei W, Wang J. One-shot versus gradual dilation technique for tract creation in percutaneous nephrolithotomy: A systematic review and meta-analysis. *Urol Res* 2013; **41**: 443-448 [PMID: 23775113]
  - 63 **Dehong C**, Liangren L, Huawei L, Qiang W. A comparison among four tract dilation methods of percutaneous nephrolithotomy: A systematic review and meta-analysis. *Urolithiasis* 2013; **41**: 523-530 [PMID: 23975408 DOI: 10.1007/s00240-013-0598-z]
  - 64 **Wezel F**, Mamoulakis C, Rioja J, Michel MS, de la Rosette J, Alken P. Two contemporary series of percutaneous tract dilation for percutaneous nephrolithotomy. *J Endourol* 2009; **23**: 1655-1661 [PMID: 19558265 DOI: 10.1089/end.2009.0213]
  - 65 **Gönen M**, Istanbuloglu OM, Cicek T, Ozturk B, Ozkardes H. Balloon dilatation versus Amplatz dilatation for nephrostomy tract dilation. *J Endourol* 2008; **22**: 901-904 [PMID: 18429681 DOI: 10.1089/end.2007.0167]
  - 66 **Safak M**, Gokus C, Soygur T. Nephrostomy tract dilation using a balloon dilator in percutaneous renal surgery: Experience with 95 cases and comparison with the fascial dilator system. *Urol Int* 2003; **71**: 382-384 [PMID: 14646437 DOI: 10.1159/000074090]
  - 67 **Al-Kandari AM**, Jabbour M, Anderson A, Shokeir AA, Smith AD. Comparative study of degree of renal trauma between Amplatz sequential fascial dilation and balloon dilation during percutaneous renal surgery in an animal model. *Urology* 2007; **69**: 586-589 [PMID: 17382184 DOI: 10.1016/j.urol.2007.01.025]
  - 68 **Chiang PH**, Su HH. Randomized and prospective trial comparing tract creation using plasma vaporization with balloon dilatation in percutaneous nephrolithotomy. *BJU Int* 2013; **112**: 89-93 [PMID: 23035747 DOI: 10.1111/j.1464-410X.2012.11507.x]
  - 69 **Gohardarakhshan RZ**, Schwartz BF, Rudnick DM, Irby PB, Stoller ML. Radially expanding single-step nephrostomy tract dilator. *Urology* 2001; **58**: 693-696 [PMID: 11711342 DOI: 10.1016/S0090-4295(01)01359-0]
  - 70 **Patil AV**. A novel 5-part Percutaneous Access Needle with Glidewire technique (5-PANG) for percutaneous nephrolithotomy: our initial experience. *Urology* 2010; **75**: 1206-1208 [PMID: 20138340 DOI: 10.1016/j.urol.2009.11.027]
  - 71 **Abraham JB**, Gamboa AJ, Finley DS, Beck SM, Lee HJ, Santos RJ, Box GN, Deane LA, Vajrta DJ, McDougall EM, Clayman RV. The UCI Seldinger technique for percutaneous renal cryoablation: protecting the tract and achieving hemostasis. *J Endourol* 2009; **23**: 43-49 [PMID: 19178171 DOI: 10.1089/end.2008.0032]
  - 72 **Muslumanoglu AY**, Tefekli A, Karadag MA, Tok A, Sari E, Berberoglu Y. Impact of percutaneous access point number and location on complication and success rates in percutaneous nephrolithotomy. *Urol Int* 2006; **77**: 340-346 [PMID: 17135785 DOI: 10.1159/000096339]
  - 73 **Williams SK**, Leveillee RJ. Management of staghorn calculus: single puncture with judicious use of the flexible nephroscope. *Curr Opin Urol* 2008; **18**: 224-228 [PMID: 18303549 DOI: 10.1097/MOU.0b013e3282f517c0]
  - 74 **Golijanin D**, Katz R, Verstandig A, Sasson T, Landau EH, Meretyk S. The supracostal percutaneous nephrostomy for treatment of staghorn and complex kidney stones. *J Endourol* 1998; **12**: 403-405 [PMID: 9847059 DOI: 10.1089/end.1998.12.403]
  - 75 **Aron M**, Yadav R, Goel R, Kolla SB, Gautam G, Hemal AK, Gupta NP. Multi-tract percutaneous nephrolithotomy for large complete staghorn calculi. *Urol Int* 2005; **75**: 327-332 [PMID: 16327300 DOI: 10.1159/000089168]
  - 76 **Ferrandino MN**, Bagrodia A, Pierre SA, Scales CD, Rampersaud E, Pearle MS, Preminger GM. Radiation exposure in the acute and short-term management of urolithiasis at 2 academic centers. *J Urol* 2009; **181**: 668-672; discussion 673 [PMID: 19100573 DOI: 10.1016/j.juro.2008.10.012]
  - 77 **Wrixon AD**. New recommendations from the International Commission on Radiological Protection--a review. *Phys Med Biol* 2008; **53**: R41-R60 [PMID: 18364557 DOI: 10.1088/0031-9155/53/8/R01]
  - 78 **Bowsher WG**, Blott P, Whitfield HN. Radiation protection in percutaneous renal surgery. *Br J Urol* 1992; **69**: 231-233 [PMID: 1568094 DOI: 10.1111/j.1464-410X.1992.tb15518.x]
  - 79 **Mancini JG**, Raymundo EM, Lipkin M, Zilberman D, Yong D, Bañez LL, Miller MJ, Preminger GM, Ferrandino MN. Factors affecting patient radiation exposure during percutaneous



- nephrolithotomy. *J Urol* 2010; **184**: 2373-2377 [PMID: 20952034 DOI: 10.1016/j.juro.2010.08.033]
- 80 **Tepeler A**, Binbay M, Yuruk E, Sari E, Kaba M, Muslumanoglu AY, Tefekli A. Factors affecting the fluoroscopic screening time during percutaneous nephrolithotomy. *J Endourol* 2009; **23**: 1825-1829 [PMID: 19811060 DOI: 10.1089/end.2009.0256]
- 81 **Dias J**, Unsworth DJ, Walker-Smith JA. Antigliadin and antireticulin antibodies in screening for coeliac disease. *Lancet* 1987; **2**: 157-158 [PMID: 2885617 DOI: 10.1007/s00345-012-0923-0]
- 82 **Yang RM**, Morgan T, Bellman GC. Radiation protection during percutaneous nephrolithotomy: a new urologic surgery radiation shield. *J Endourol* 2002; **16**: 727-731 [PMID: 12542875 DOI: 10.1089/08927790260472872]
- 83 **Elkoushy MA**, Shahrour W, Andonian S. Pulsed fluoroscopy in ureteroscopy and percutaneous nephrolithotomy. *Urology* 2012; **79**: 1230-1235 [PMID: 22465084 DOI: 10.1016/j.urology.2012.01.027]
- 84 **Lipkin ME**, Mancini JG, Zilberman DE, Raymundo ME, Yong D, Ferrandino MN, Miller MJ, Yoshizumi TT, Preminger GM. Reduced radiation exposure with the use of an air retrograde pyelogram during fluoroscopic access for percutaneous nephrolithotomy. *J Endourol* 2011; **25**: 563-567 [PMID: 21426236 DOI: 10.1089/end.2010.0431]
- 85 **Zeng G**, Zhao Z, Zhong W, Wu K, Chen W, Wu W, Xiao C, Liu Y. Evaluation of a novel fascial dilator modified with scale marker in percutaneous nephrolithotomy for reducing the X-ray exposure: a randomized clinical study. *J Endourol* 2013; **27**: 1335-1340 [PMID: 23527890 DOI: 10.1089/end.2012.0671]
- 86 **El-Nahas AR**, Shokeir AA, El-Assmy AM, Shoma AM, Eraky I, El-Kenawy MR, El-Kappany HA. Colonic perforation during percutaneous nephrolithotomy: study of risk factors. *Urology* 2006; **67**: 937-941 [PMID: 16635515 DOI: 10.1016/j.urology.2005.11.025]
- 87 **Korkes F**, Lopes Neto AC, Lucio J, Bezerra CA, Wroklawski ER. Management of colon injury after percutaneous renal surgery. *J Endourol* 2009; **23**: 569-573 [PMID: 19335215 DOI: 10.1089/end.2008.0506]
- 88 **Maheshwari PN**. Retro-renal colon: Is it an acquired anomaly? *J Endourol* 2011; **28**: A16U
- 89 **El-Nahas AR**, Mansour AM, Ellaithy R, Abol-Enein H. Case report: conservative treatment of liver injury during percutaneous nephrolithotomy. *J Endourol* 2008; **22**: 1649-1652 [PMID: 18657040]
- 90 **Shah HN**, Hegde SS, Mahajan AP, Sodha H, Shah R, Bansal M. Splenic injury: rare complication of percutaneous nephrolithotomy: report of two cases with review of literature. *J Endourol* 2007; **21**: 919-922 [PMID: 17867954]
- 91 **Maheshwari PN**, Mane DA, Pathak AB. Management of pleural injury after percutaneous renal surgery. *J Endourol* 2009; **23**: 1769-1772 [PMID: 19785558 DOI: 10.1089/end.2009.1549]
- 92 **Sukumar S**, Nair B, Ginil KP, Sanjeevan KV, Sanjay BH. Supracostal access for percutaneous nephrolithotomy: less morbid, more effective. *Int Urol Nephrol* 2008; **40**: 263-267 [PMID: 17899435 DOI: 10.1007/s11255-007-9270-2]
- 93 **Stening SG**, Bourne S. Supracostal percutaneous nephrolithotomy for upper pole caliceal calculi. *J Endourol* 1998; **12**: 359-362 [PMID: 9726403 DOI: 10.1089/end.1998.12.359]
- 94 **Finelli A**, Honey RJ. Thoracoscopy-assisted high intercostal percutaneous renal access. *J Endourol* 2001; **15**: 581-584; discussion 584-585 [PMID: 11552780 DOI: 10.1089/089277901750426337]
- 95 **Ogan K**, Corwin TS, Smith T, Watumull LM, Mullican MA, Cadeddu JA, Pearle MS. Sensitivity of chest fluoroscopy compared with chest CT and chest radiography for diagnosing hydropneumothorax in association with percutaneous nephrostolithotomy. *Urology* 2003; **62**: 988-992 [PMID: 14665341 DOI: 10.1016/j.urology.2003.07.024]
- 96 **Zagoria RJ**, Dyer RB. Do's and don't's of percutaneous nephrostomy. *Acad Radiol* 1999; **6**: 370-377 [PMID: 10376069 DOI: 10.1016/S1076-6332(99)80233-5]
- 97 **Grasso M**, Lang G, Taylor FC. Flexible ureteroscopically assisted percutaneous renal access. *Tech Urol* 1995; **1**: 39-43 [PMID: 9118366]
- 98 **Giannakopoulos S**, Bantis A, Kalaitzis C, Touloupidis S. Use of an angiographic catheter to facilitate fluoroscopy-guided percutaneous renal access in cases with diffuse contrast extravasation. *J Endourol* 2010; **24**: 1575-1578 [PMID: 20818986 DOI: 10.1089/end.2010.0019]
- 99 **Rais-Bahrami S**, Friedlander JI, Duty BD, Okeke Z, Smith AD. Difficulties with access in percutaneous renal surgery. *Ther Adv Urol* 2011; **3**: 59-68 [PMID: 21869906 DOI: 10.1177/1756287211400661]
- 100 **Lojanapiwat B**. The ideal puncture approach for PCNL: Fluoroscopy, ultrasound or endoscopy? *Indian J Urol* 2013; **29**: 208-213 [PMID: 24082442 DOI: 10.4103/0970-1591.117284]
- 101 **Skolarikos A**, Alivizatos G, de la Rosette JJ. Percutaneous nephrolithotomy and its legacy. *Eur Urol* 2005; **47**: 22-28 [PMID: 15582245 DOI: 10.1016/j.eururo.2004.08.009]
- 102 **Lezrek M**, Bazine K, Asseban M, Ammani A, Moufid K, Beddouch A, Alami M. Technique to overcome renal mobility during percutaneous tract dilatation: bi-prong forceps renal parenchyma dissection. *BJU Int* 2013; **112**: 697-702 [PMID: 23924426]
- 103 **Maheshwari PN**, Andankar MG, Bansal M. Nephrostomy tube after percutaneous nephrolithotomy: large-bore or pigtail catheter? *J Endourol* 2000; **14**: 735-737; discussion 737-738 [PMID: 11110567 DOI: 10.1089/end.2000.14.735]

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## Robotics and surgery: A sustainable relationship?

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### Abstract

Robotic surgery is increasingly being employed to overcome the disadvantages associated with use of conventional techniques such as laparoscopy. However, despite significant promise, there are some clear disadvantages and robust evidence base supporting the use of robotic assistance remains lacking. In this paper, the advantages and drivers for robotics will be discussed, its drawbacks and its future role in surgery.

**Key words:** Robotics; Surgery; Simulation; Patient safety

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**Core tip:** Robotic technology is increasingly being employed in surgery to overcome the disadvantages associated with use of conventional techniques such as laparoscopy. However, despite significant promise, robust evidence base supporting the use of robotic assistance remains lacking. Prospective, multicentre randomised controlled trials to evaluate efficacy, long-term outcomes, safety and cost are the next steps before widespread uptake of this technology to treat patients. Moreover, with the unprecedented need for patient safety, it is imperative that adequate training and assessment strategies are in place to bridge the gap between conventional techniques and robotic surgery without harm to patients.

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### INTRODUCTION

Robotic surgery is increasingly being implemented to overcome drawbacks associated with the use of conventional techniques such as laparoscopy, especially in complex procedures. However, despite holding significant promise, robotic surgery is associated with some clear disadvantages and robust evidence base supporting robotic assistance remains lacking<sup>[1]</sup>.

The introduction of minimally invasive techniques to general surgery has been described as "the most dramatic change in surgery since the introduction of anaesthesia"<sup>[2]</sup>. This has led to many procedures being performed exclusively *via* the laparoscopic approach, such as a cholecystectomy. Reasons include reduced blood loss and post-operative pain, reduced risk of infection, reduced length of hospital stay and faster return to daily activities<sup>[3]</sup>. However, these superior

results are only when the initial learning curve has been taken into account.

Laparoscopic surgery is associated with several challenges. Disadvantages and complications have been well documented<sup>[4]</sup>. Long, rigid instruments amplify tremor, reduce range of motion and degrees of freedom. This is exacerbated by the fulcrum effect whereby instrument tips move in a direction opposite to those of surgeon's hands<sup>[5]</sup>. Loss of 3-dimensional (3D) vision and having to view a 2-dimensional image, not directly under the control of surgeon, enhances these difficulties by leading to loss of traditional eye-hand target axis<sup>[6]</sup>. The laparoscopic technique is associated with poor ergonomics and health problems in surgeons such as nerve injuries<sup>[7]</sup>. Robotic systems, such as the da Vinci, have thus emerged to overcome few of these limitations.

The 3D, high-definition imaging of robotic technology facilitates stereotactic vision of the operation field and makes depth perception possible<sup>[8]</sup>. The camera is surgeon-controlled and the area of interest can be magnified up to 10 times. The surgeon's hand movements can be scaled (5:1, 3:1, or 1:1) so that large hand movements are translated into smaller movements inside the patient<sup>[9]</sup>. Combined with tremor abolition, this facilitates precise surgical manoeuvres. Endowrist instrumentation provides 7 degrees of freedom and improves range of motion, enhancing dexterity, comparable to that attained in open procedures<sup>[10]</sup>. The surgeon's comfort is increased by the ergonomic sitting position, reducing fatigue (both physical and cognitive) due to exhausting positions or movements often observed in conventional laparoscopy<sup>[11]</sup>. The intuitive movements in robotic surgery can potentially shorten the learning curve compared to conventional laparoscopy<sup>[12]</sup>. Thus, less experienced laparoscopic surgeons may acquire skills to conduct robotic surgeries in a relatively shorter time period compared to attaining corresponding proficiency in conventional laparoscopy. Significant progress in robotic applications has been in procedures that cannot be performed by a laparoscopic approach, *i.e.*, cardiac and endovascular surgery.

## CARDIAC SURGERY

A Total Endoscopic Coronary Artery Bypass (TECAB) can now be performed using the robotic slave system, da Vinci, from the Left Internal Mammary Artery to the Left Anterior Descending artery without the need for a sternotomy<sup>[13]</sup>. Successful results have been reported by several groups as a result of reduced post-operative pain, better cosmesis and faster healing due to lack of a sternotomy incision<sup>[14]</sup>. Procedures requiring extreme precision or fine visualisation, such as coronary anastomosis are facilitated by the high magnification and tremor-free, precise microinstrumentation<sup>[15]</sup>. Greater patient satisfaction is also reported<sup>[16]</sup>. Off-

pump procedures (*i.e.*, on a beating heart) avoid complications of cardiopulmonary bypass and are associated with a lower incidence of atrial fibrillation, stroke and death in the elderly<sup>[17]</sup>. Robotic surgery is also useful for mitral valve reconstruction. 3D visualisation allows good view of the ventricle needed for suturing in chordal reconstruction<sup>[18]</sup>. Greater range of motion facilitates the complex cutting and needle loading angles in the confined space of the left atrium<sup>[18]</sup>.

However, there are disadvantages. Robot-assisted TECAB is a technically demanding and time-consuming procedure. It is associated with a significant learning curve<sup>[19]</sup>. Nevertheless, it represents a feasible alternative to conventional coronary artery bypass<sup>[20]</sup>.

## ENDOVASCULAR SURGERY

Another emerging domain of robotic surgery is that of endovascular robotics. The conventional endovascular catheters present several limitations within the vascular tree. These include their small range of shapes and sizes, difficulty in maneuvering the tip with the lack of stability<sup>[21]</sup>. Hence, interventionalists have to frequently change catheters and this presents a major risk of vessel trauma or distal embolization as a result of alteration of guidewire position<sup>[21]</sup>. This is especially critical in the aortic arch, where stroke, as a result of cerebral embolisation, may occur<sup>[21]</sup>.

Riga *et al*<sup>[22]</sup> demonstrated that Endovascular Aneurysm Repair using a robotically steerable catheter system is feasible and may improve catheter maneuverability, stability and precision. Pre-shaped conventional catheters can rotate around one axis only, presenting a major drawback when fine and controlled movements are required in multiple planes<sup>[22]</sup>. Conversely, a steerable multidirectional catheter may overcome this hurdle and may be especially useful with regards to anatomically difficult cannulation in fenestrated stent-grafting<sup>[23]</sup>. This system also minimises operator radiation exposure, as the workstation is located outside the endovascular suite and away from the radiation source. Robotic endovascular catheters may lead to improved accuracy, reduce time and minimise radiation exposure in complex vascular procedures in particular<sup>[23]</sup>. Moreover, robotic endovascular catheters have been demonstrated to lead to a statistically significant faster skill acquisition in novice surgeons<sup>[24]</sup>. Hence, there is a potential to shorten the learning curve so that trainees can attempt more complex endovascular procedures earlier and with a greater degree of safety<sup>[24]</sup>. Yet, transferability of these findings to the operating room (OR) is debatable.

## DRAWBACKS AND THE FUTURE

Despite the numerous advantages, robotics in surgery has drawbacks that hinder the widespread

**Table 1 Drawbacks associated with robotic surgery**

Drawback	Discussion
Cost	The da Vinci system costs approximately \$1.5 million with maintenance fees of about \$150000 per year <sup>[43,44]</sup> . Likewise, robotic endovascular catheter systems are expensive, have high maintenance costs, with the additional cost of disposable catheters. However, there is no conclusive data regarding the cost-effectiveness of these robotic systems. Moreover, an economic model, with quality of life adjustment, has not been performed for any of the robotic systems <sup>[44]</sup>
Evidence	Currently, the evidence for robotic surgery's efficacy and safety is largely from retrospective studies often with small sample sizes or from an institution's initial cases/experiences, where the surgeon may be at the start of his/her learning curve <sup>[44]</sup> . Hence, conclusions about safety and efficacy must be interpreted with caution
Preparation, floor space and emergencies	The Theatre team must also be trained with the device set-up including troubleshooting problems that may arise during operations. Hence, the robotic surgery venture is likely a time, cost and resource-intensive process <sup>[45]</sup> . Moreover, considerable floor space is needed, with bulky instruments; this may be problematic and considerable cost may be incurred for renovations before robotic surgery can be employed. Furthermore, in an emergency, there may be a delay in converting to an open procedure since the bulky instruments cannot be as easily removed as in conventional laparoscopy <sup>[44]</sup>
Unproven efficacy	Current evidence base for efficacy of robotic surgery is mainly from small, retrospective studies. Prospective, multicentre randomized clinical trials to evaluate safety, efficacy, long term outcomes and cost analysis are required to prove that robotic assistance is indeed superior to conventional techniques before its widespread use

**Table 2 Definitions of validity and reliability**

Type	Definition
Face Validity	Extent to which the simulator resembles real life scenarios
Content Validity	Extent to which the domain that is being measured is being measured by the simulator/assessment tool
Construct Validity	Extent to which a simulator measures the trait it purports to measure
Concurrent Validity	Extent to which the results of the assessment tool correlate with the gold standard for that domain
Predictive Validity	Ability of the simulator to predict future performance
Test-Retest Reliability	Measure of a test to generate similar results when applied at two different points
Inter-Rater Reliability	Measure of agreement between two or more observers when rating an individual's performance

implementation of its usage (Table 1). In particular, the evidence base supporting robotic assistance remains lacking<sup>[1]</sup>. This extends beyond the examples provided above. A robotic prostatectomy is now the standard of care in many centres; despite only one RCT and substantial publication and selection bias, the results have showed no significant improvement in patient morbidity compared with conventional laparoscopy<sup>[25]</sup>. Likewise, a Cochrane review showed no differences in safety and efficacy for benign gynaecological robotic surgery compared to conventional laparoscopy<sup>[26]</sup>.

Results from high quality, prospective, multi-centre randomized clinical trials (RCTs) are urgently required to evaluate the true efficacy of robotic surgery. Enhanced patient care may justify any higher costs. For surgeons uncomfortable with advanced conventional techniques, robotic surgery may reduce the time for them to reach procedure proficiency. For experienced surgeons, robotic surgery may enhance precision and decrease physical and mental workload.

With an unprecedented need for patient safety<sup>[27]</sup>, it is imperative that adequate training and assessment strategies are in place to bridge the gap between conventional techniques and robotic surgery without harm to patients. This is especially important now with reduced working hours and training opportunities following calmanisation and introduction of the European Working Time Directive<sup>[28]</sup>. Possible avenues include: (1) Virtual Reality (VR) simulation; (2) Use of dual consoles;

and (3) Training courses.

## VR SIMULATION

VR simulation has been well established for conventional laparoscopy and has shown to improve skill transfer to the operating room<sup>[29,30]</sup>. However, its effectiveness in robotic surgery is less clear<sup>[31]</sup>. Before a simulator is used, it must fulfil a criterion with regards to validity and reliability (Table 2)<sup>[31]</sup>. Indeed, a study by Hung *et al*<sup>[32,33]</sup> showed that the da Vinci Skills Simulator demonstrated content, face and construct validity. The performance of the expert group was superior to intermediate/novice group when evaluating parameters such as overall score, motion economy and time to completion<sup>[32]</sup>. Specific proficiency-based curricula need to be developed in order to provide structured training with in built measures of assessment. However, while VR simulation for Robotic Surgical Training is a promising tool, data on skills transfer to the operating room is still lacking and further work is required before we can draw any firm conclusions about its efficacy in training. Another promising strategy is use of a dual console.

## DUAL CONSOLE

The dual console allows collaboration between the trainee and an experienced mentor<sup>[31]</sup>. There are two



collaborative modes: (1) "Swap mode" enables the experienced surgeon and the trainee to operate in parallel and switch control of the robotic arms; this facilitates parts of the operation requiring multiple hands, for example vessel isolation<sup>[31,33]</sup>; and (2) "Nudge mode" enables trainee and mentor to share the two robotic arms which is useful during key parts of the operation whereby the mentor can guide the hands of the trainee<sup>[31,33,34]</sup>. Marengo *et al*<sup>[35]</sup> suggested that use of dual consoles might shorten the learning curve and increase trainees' confidence in performing procedures. However, the data for the efficacy of dual consoles is scarce and prospective, RCTs are required to evaluate their true efficacy in surgical training<sup>[31]</sup>.

## TRAINING COURSES

Training courses, using animal, inanimate or cadaveric models have shown promise<sup>[31]</sup>. Assessment parameters include time to setup and operate, complications, errors and quality as determined by the Objective Structured Assessment of Technical Skills score<sup>[34,36]</sup>. Dulan *et al*<sup>[37]</sup> have developed a proficiency-based robotic training program that demonstrates construct and content validity as well as feasibility. Further validation of such curricula should be encouraged since we know that for conventional laparoscopy, achieving proficiency ascertains whether a surgeon has the aptitude to perform a procedure; this is not related to the length of training<sup>[38]</sup>. Aggarwal *et al*<sup>[39]</sup> demonstrated that a proficiency-based curriculum for laparoscopic cholecystectomy could shorten the learning curve resulting in faster skill acquisition. Moreover, such curricula for robotic surgery may provide the opportunity to exercise deliberate practice that has been regarded as a key practice to enhance and acquire "expert performance"<sup>[40,41]</sup>. And crucially, proficiency-based curricula may allow standardisation in training and assessment<sup>[39]</sup>.

Finally, future development and innovation in more advanced technology for procedures that are challenging to perform with conventional as well as current robotic technology is warranted with the ultimate aim of improving patient outcome. The new imaging-sensing-navigated, kinematically enhanced robot, a flexible-access robot with integrated multimodal and multi-scale sensing, can enable the surgeon to guide tools into regions of the body that are difficult to access with the current technology<sup>[42]</sup>. It has already shown promising results *in vivo*, with clinical translation planned in the next couple of years<sup>[42]</sup>.

## CONCLUSION

Like when laparoscopic surgery was introduced, establishing the role of robotic surgery will take time and to ascertain which patients are most likely to benefit from it. Prospective, multicentre randomised controlled trials to evaluate efficacy, long-term outcomes, safety and cost are the next steps before widespread uptake

of this technology to treat patients.

## REFERENCES

- 1 Sodergren MH, Darzi A. Robotic cancer surgery. *Br J Surg* 2013; **100**: 3-4 [PMID: 23132653 DOI: 10.1002/bjs.8972]
- 2 Royston CM, Lansdown MR, Brough WA. Teaching laparoscopic surgery: the need for guidelines. *BMJ* 1994; **308**: 1023-1025 [PMID: 8167516 DOI: 10.1136/bmj.308.6935.1023]
- 3 Agha R, Muir G. Does laparoscopic surgery spell the end of the open surgeon? *J R Soc Med* 2003; **96**: 544-546 [PMID: 14594961 DOI: 10.1258/jrsm.96.11.544]
- 4 Duca S, Bălă O, Al-Hajjar N, Lancu C, Puia IC, Munteanu D, Graur F. Laparoscopic cholecystectomy: incidents and complications. A retrospective analysis of 9542 consecutive laparoscopic operations. *HPB (Oxford)* 2003; **5**: 152-158 [PMID: 18332976 DOI: 10.1080/13651820304298]
- 5 Bittner JG, Hathaway CA, Brown JA. [In Process Citation]. *J Minim Access Surg* 2008; **4**: 31-38 [PMID: 19547678 DOI: 10.4103/0972-9941.41938]
- 6 Aggarwal R, Hance J, Darzi A. Robotics and surgery: a long-term relationship? *Int J Surg* 2004; **2**: 106-109 [PMID: 17462231 DOI: 10.1016/S1743-9191(06)60055-1]
- 7 Supe AN, Kulkarni GV, Supe PA. Ergonomics in laparoscopic surgery. *J Minim Access Surg* 2010; **6**: 31-36 [PMID: 20814508 DOI: 10.4103/0972-9941.65161]
- 8 Mylonas GP, Darzi A, Yang GZ. Gaze-contingent control for minimally invasive robotic surgery. *Comput Aided Surg* 2006; **11**: 256-266 [PMID: 17127651 DOI: 10.3109/10929080600971344]
- 9 Prasad SM, Prasad SM, Maniar HS, Chu C, Schuessler RB, Damiano RJ. Surgical robotics: impact of motion scaling on task performance. *J Am Coll Surg* 2004; **199**: 863-868 [PMID: 15555968 DOI: 10.1016/j.jamcollsurg.2004.08.027]
- 10 Kumar R, Hemal AK. Emerging role of robotics in urology. *J Minim Access Surg* 2005; **1**: 202-210 [PMID: 21206664 DOI: 10.4103/0972-9941.19268]
- 11 van der Schatte Olivier RH, Van't Hullenaar CD, Ruurda JP, Broeders IA. Ergonomics, user comfort, and performance in standard and robot-assisted laparoscopic surgery. *Surg Endosc* 2009; **23**: 1365-1371 [PMID: 18855053 DOI: 10.1007/s00464-008-0184-6]
- 12 Chandra V, Nehra D, Parent R, Woo R, Reyes R, Hernandez-Boussard T, Dutta S. A comparison of laparoscopic and robotic assisted suturing performance by experts and novices. *Surgery* 2010; **147**: 830-839 [PMID: 20045162 DOI: 10.1016/j.surg.2009.11.002]
- 13 Kappert U, Schneider J, Cichon R, Gulielmos V, Schade I, Nicolai J, Schueler S. Closed chest totally endoscopic coronary artery bypass surgery: fantasy or reality? *Curr Cardiol Rep* 2000; **2**: 558-563 [PMID: 11060584 DOI: 10.1007/s11886-000-0042-1]
- 14 Bonatti J, Schachner T, Bonaros N, Lehr EJ, Zimrin D, Griffith B. Robotically assisted totally endoscopic coronary bypass surgery. *Circulation* 2011; **124**: 236-244 [PMID: 21747068 DOI: 10.1161/CIRCULATIONAHA.110.985267]
- 15 Boyd WD, Desai ND, Kiai B, Rayman R, Menkis AH, McKenzie FN, Novick RJ. A comparison of robot-assisted versus manually constructed endoscopic coronary anastomosis. *Ann Thorac Surg* 2000; **70**: 839-842; discussion 842-843 [PMID: 11016320 DOI: 10.1016/S0003-4975(00)01738-0]
- 16 Deeba S, Aggarwal R, Sains P, Martin S, Athanasiou T, Casula R, Darzi A. Cardiac robotics: a review and St. Mary's experience. *Int J Med Robot* 2006; **2**: 16-20 [PMID: 17520609 DOI: 10.1002/rcs.76]
- 17 Panesar SS, Athanasiou T, Nair S, Rao C, Jones C, Nicolaou M, Darzi A. Early outcomes in the elderly: a meta-analysis of 4921 patients undergoing coronary artery bypass grafting--comparison between off-pump and on-pump techniques. *Heart* 2006; **92**: 1808-1816 [PMID: 16775087 DOI: 10.1136/hrt.2006.088450]
- 18 Atluri P, Woo YJ. Minimally invasive robotic mitral valve surgery. *Expert Rev Med Devices* 2011; **8**: 115-120 [PMID: 21158546 DOI: 10.1586/erd.10.66]
- 19 Bonatti J, Schachner T, Bernecker O, Chevtchik O, Bonaros N, Ott

- H, Friedrich G, Weidinger F, Laufer G. Robotic totally endoscopic coronary artery bypass: program development and learning curve issues. *J Thorac Cardiovasc Surg* 2004; **127**: 504-510 [PMID: 14762361 DOI: 10.1016/j.jtcvs.2003.09.005]
- 20 **Acharya MN**, Ashrafi H, Athanasiou T, Casula R. Is totally endoscopic coronary artery bypass safe, feasible and effective? *Interact Cardiovasc Thorac Surg* 2012; **15**: 1040-1046 [PMID: 22976997 DOI: 10.1093/icvts/ivs395]
- 21 **di Marco A**, Riga C, Hamady M, Cheshire N, Bicknell C. Robotic and Navigational Technologies in Endovascular Surgery. *Vascular Disease Management* 2010; **7**: E15-E19
- 22 **Riga C**, Bicknell C, Cheshire N, Hamady M. Initial clinical application of a robotically steerable catheter system in endovascular aneurysm repair. *J Endovasc Ther* 2009; **16**: 149-153 [PMID: 19456202 DOI: 10.1583/08-2651.1]
- 23 **Riga CV**, Cheshire NJ, Hamady MS, Bicknell CD. The role of robotic endovascular catheters in fenestrated stent grafting. *J Vasc Surg* 2010; **51**: 810-819; discussion 819-820 [PMID: 20347674 DOI: 10.1016/j.jvs.2009.08.101]
- 24 **Riga CV**, Bicknell CD, Sidhu R, Cochenne F, Normahani P, Chadha P, Kashef E, Hamady M, Cheshire NJ. Advanced catheter technology: is this the answer to overcoming the long learning curve in complex endovascular procedures. *Eur J Vasc Endovasc Surg* 2011; **42**: 531-538 [PMID: 21388839 DOI: 10.1016/j.ejvs.2011.02.004]
- 25 **Ficarra V**, Novara G, Artibani W, Cestari A, Galfano A, Graefen M, Guazzoni G, Guillonnet B, Menon M, Montorsi F, Patel V, Rassweiler J, Van Poppel H. Retropubic, laparoscopic, and robot-assisted radical prostatectomy: a systematic review and cumulative analysis of comparative studies. *Eur Urol* 2009; **55**: 1037-1063 [PMID: 19185977 DOI: 10.1016/j.eururo.2009.01.036]
- 26 **Liu H**, Lu D, Wang L, Shi G, Song H, Clarke J. Robotic surgery for benign gynaecological disease. *Cochrane Database Syst Rev* 2012; **2**: CD008978 [PMID: 22336855 DOI: 10.1002/14651858.CD008978.pub2]
- 27 **Kohn L**. To err is human: an interview with the Institute of Medicine's Linda Kohn. *Jt Comm J Qual Improv* 2000; **26**: 227-234 [PMID: 10749007]
- 28 **Chikwe J**, de Souza AC, Pepper JR. No time to train the surgeons. *BMJ* 2004; **328**: 418-419 [PMID: 14976074 DOI: 10.1136/bmj.328.7437.418]
- 29 **Seymour NE**, Gallagher AG, Roman SA, O'Brien MK, Bansal VK, Andersen DK, Satava RM. Virtual reality training improves operating room performance: results of a randomized, double-blinded study. *Ann Surg* 2002; **236**: 458-463; discussion 463-464 [PMID: 12368674 DOI: 10.1097/00000658-200210000-00008]
- 30 **Grantcharov TP**, Kristiansen VB, Bendix J, Bardram L, Rosenberg J, Funch-Jensen P. Randomized clinical trial of virtual reality simulation for laparoscopic skills training. *Br J Surg* 2004; **91**: 146-150 [PMID: 14760660 DOI: 10.1002/bjs.4407]
- 31 **Buchs NC**, Pugin F, Volonté F, Morel P. Learning tools and simulation in robotic surgery: state of the art. *World J Surg* 2013; **37**: 2812-2819 [PMID: 23640724 DOI: 10.1007/s00268-013-2065-y]
- 32 **Hung AJ**, Zehnder P, Patil MB, Cai J, Ng CK, Aron M, Gill IS, Desai MM. Face, content and construct validity of a novel robotic surgery simulator. *J Urol* 2011; **186**: 1019-1024 [PMID: 21784469 DOI: 10.1016/j.juro.2011.04.064]
- 33 **Hung AJ**, Patil MB, Zehnder P, Cai J, Ng CK, Aron M, Gill IS, Desai MM. Concurrent and predictive validation of a novel robotic surgery simulator: a prospective, randomized study. *J Urol* 2012; **187**: 630-637 [PMID: 22177176 DOI: 10.1016/j.juro.2011.09.154]
- 34 **Hanly EJ**, Miller BE, Kumar R, Hasser CJ, Coste-Maniere E, Talamini MA, Aurora AA, Schenkman NS, Marohn MR. Mentoring console improves collaboration and teaching in surgical robotics. *J Laparoendosc Adv Surg Tech A* 2006; **16**: 445-451 [PMID: 17004866 DOI: 10.1089/lap.2006.16.445]
- 35 **Marengo F**, Larraín D, Babilonti L, Spinillo A. Learning experience using the double-console da Vinci surgical system in gynecology: a prospective cohort study in a University hospital. *Arch Gynecol Obstet* 2012; **285**: 441-445 [PMID: 21779771 DOI: 10.1007/s00404-011-2005-8]
- 36 **Narazaki K**, Oleynikov D, Stergiou N. Robotic surgery training and performance: identifying objective variables for quantifying the extent of proficiency. *Surg Endosc* 2006; **20**: 96-103 [PMID: 16374675 DOI: 10.1007/s00464-005-3011-3]
- 37 **Dulan G**, Rege RV, Hogg DC, Gilberg-Fisher KM, Arain NA, Tesfay ST, Scott DJ. Developing a comprehensive, proficiency-based training program for robotic surgery. *Surgery* 2012; **152**: 477-488 [PMID: 22938907 DOI: 10.1016/j.surg.2012.07.028]
- 38 **Aggarwal R**, Darzi A, Grantcharov TP. Re: A systematic review of skills transfer after surgical simulation training. *Ann Surg* 2008; **248**: 690-691; author reply 691 [PMID: 18936587 DOI: 10.1097/SLA.0b013e3181884320]
- 39 **Aggarwal R**, Grantcharov T, Moorthy K, Milland T, Papasavas P, Dosis A, Bello F, Darzi A. An evaluation of the feasibility, validity, and reliability of laparoscopic skills assessment in the operating room. *Ann Surg* 2007; **245**: 992-999 [PMID: 17522527]
- 40 **Crochet P**, Aggarwal R, Dubb SS, Ziprin P, Rajaretnam N, Grantcharov T, Ericsson KA, Darzi A. Deliberate practice on a virtual reality laparoscopic simulator enhances the quality of surgical technical skills. *Ann Surg* 2011; **253**: 1216-1222 [PMID: 21516035 DOI: 10.1097/SLA.0b013e3182197016]
- 41 **Ericsson KA**. Deliberate practice and acquisition of expert performance: a general overview. *Acad Emerg Med* 2008; **15**: 988-994 [PMID: 18778378 DOI: 10.1111/j.1553-2712.2008.00227.x]
- 42 **Monaco A**. The Next Generation of Surgical Robots. The Institute. December 7 2012. Available from: URL: <http://theinstitute.ieee.org/technology-focus/technology-topic/the-next-generation>
- 43 **Monod P**. Financial aspects, or how to use a robot assistance without losing money. Perspectives from private practice. *J Visc Surg* 2011; **148**: e22-e26 [PMID: 21967778 DOI: 10.1016/j.jvisurg.2011.04.005]
- 44 **De Wilde RL**, Herrmann A. Robotic surgery - advance or gimmick? *Best Pract Res Clin Obstet Gynaecol* 2013; **27**: 457-469 [PMID: 23357200 DOI: 10.1016/j.bpobgyn.2012.12.005]
- 45 **Lanfranco AR**, Castellanos AE, Desai JP, Meyers WC. Robotic surgery: a current perspective. *Ann Surg* 2004; **239**: 14-21 [PMID: 14685095 DOI: 10.1097/01.sla.0000103020.19595.7d]

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## Endoscopic treatment of orbital tumors

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to intraconal lesions may also require deinsertion of extraocular muscles, with subsequent impact on extraocular mobility. Recently, minimally invasive techniques have been proposed as valid alternative to external approaches for selected orbital lesions. Among them, transnasal endoscopic approaches, "pure" or combined with external approaches, have been reported, especially for intraconal lesions located inferiorly and medially to the optic nerve. The avoidance of muscle detachment and the shortness of the surgical intraorbital trajectory makes endoscopic approach less invasive, thus minimizing tissue damage. Endoscopic surgery decreases the recovery time and improves the cosmetic outcome not requiring skin incisions. The purpose of this study is to review and discuss the current surgical techniques for orbital tumors removal, focusing on endoscopic approaches to the orbit and outlining the key anatomic principles to follow for safe tumor resection.

**Key words:** Orbit; Orbital tumor; Endoscopy; Surgery; Approach

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**Core tip:** Recently, minimally invasive techniques have been proposed as valid alternative to external orbital and transcranial approaches for selected orbital lesions. Among them, transnasal endoscopic approaches, "pure" or combined with external approaches, have been reported, especially for intraconal lesions located inferiorly and medially to the optic nerve. Herein we review and discuss the current surgical techniques for orbital tumors removal, focusing on endoscopic approaches to the orbit and outlining the key anatomic principles to follow for safe tumor resection.

### Abstract

Different orbital and transcranial approaches are performed in order to manage orbital tumors, depending on the location and size of the lesion within the orbit. These approaches provide a satisfactory view of the superior and lateral aspects of the orbit and the optic canal but involve risks associated with their invasiveness because they require significant displacement of orbital structures. In addition, external approaches

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## INTRODUCTION

Orbital tumors encompass a broad spectrum of benign and malignant lesions intrinsic to the orbit, like cavernous hemangiomas, schwannomas, hemangiopericytomas, and tumors starting from the skin, sinuses, nose, cranial bones and cerebral parenchima with secondary orbital invasion. Cavernous hemangiomas are the most frequent intraorbital primary tumors in adults, representing 4% of all orbital tumors and 9%-13% of all intracranial cavernous hemangiomas<sup>[1]</sup>.

Exact location within the orbital cavity and size of tumors are crucial elements involved in the surgical planning.

External surgical approaches to the orbit have already extensively been described. Lateral orbitotomy or the transconjunctival approach are usually performed for the removal of small tumors located on the temporal compartment or on the orbital base; supraorbital approach allows the resection of lesions located dorsolaterally; transcranial approaches, like pterional approach, are indicated for large tumors even located medially to the optic nerve.

Recently, minimally invasive techniques have been proposed as valid alternative to external approaches for selected orbital lesions. Norris and Cleasby firstly described the use of the endoscope in orbital surgery in 1981<sup>[2]</sup>. Endoscopic management of orbital lesions was initially reported in 1985 by Norris *et al.*<sup>[3]</sup>. Thereafter different endoscopic approaches have been described for orbital tumors removal with the aim of reducing the morbidity rate related to a more significant tissue manipulation while preserving cosmesis. Transnasal endoscopic approaches are also well established for different non-tumoral conditions like Graves' ophthalmopathy<sup>[4]</sup>, medial wall fracture<sup>[5]</sup> and traumatic optic neuropathy unresponsive to steroids<sup>[6]</sup>.

Several authors have reported on different transnasal endoscopic approaches for orbital tumors removal, especially for intraconal lesions located inferiorly and medially to the optic nerve. Most surgeons performed "pure" endonasal approaches; some others described combined "open" orbital and endoscopic surgery<sup>[7]</sup>. Mir-Salim *et al.*<sup>[8]</sup> removed an intraconal cavernous hemangioma through an endonasal transtethmoidal route with the aid of microscope. The expanded endonasal approach allows the removal of all types of skull base tumors, including posterior and medial orbital lesions<sup>[9]</sup>.

Very recently, direct transorbital endoscopic approaches have been described for posterior lateral orbital tumors removal<sup>[10]</sup>.

The aim of the present study is to review and discuss the current surgical techniques for orbital

tumors removal, focusing on endoscopic approaches to the orbit.

## OPERATIVE TECHNIQUES

### External approaches

Lateral orbitotomy, providing a wide exposure of the lateral orbital compartment, is universally indicated for extra- and intraconal lesions situated therein, such as pleomorphic adenomas and cavernous hemangiomas<sup>[11-15]</sup>.

The transconjunctival approach is restricted to smaller basal and medial intra- and extraconal tumors, such as cavernous hemangiomas, schwannomas, hemangiopericytomas, and isolated neurofibromas<sup>[15,16]</sup>. This approach implies incision of the conjunctiva inferiorly along the corneal edge and caudal opening of the flap<sup>[17,18]</sup>.

The supraorbital approach *via* eyebrow incision is indicated for lesions located superiorly to the optic nerve<sup>[19]</sup>. This approach is more suited to large extraconal lesions in which added exposure is needed<sup>[15]</sup>.

The pterional approach offers excellent exposure of the posterior orbit and wide visualization of the superior orbital fissure and the anterior temporal fossa, also allowing access to the upper part of the medial orbit<sup>[19,20]</sup>. The contralateral pterional was considered suitable for lesions located medially and inferiorly to the optic nerve in the posterior intraconal space<sup>[20]</sup>.

### Endonasal microsurgical approach

Mir-Salim *et al.*<sup>[8]</sup> described an endonasal transtethmoidal approach performed with the aid of microscope in order to remove a cavernous hemangioma. They performed ethmoidectomy and then resected the lamina papyracea between the sphenoid sinus wall, skull base and ethmoid. After mobilization of the medial rectus muscle, the cavernoma was removed under microscopic control.

### Endonasal endoscopic approaches

Transnasal endoscopic approach is indicated for intraconal lesions located inferiorly and medially to the optic nerve, especially cavernous hemangiomas, which can be easily manipulated with low risk of rupture thus resulting ideal for the transnasal management<sup>[21]</sup>.

Usually sphenoethmoidectomy is performed by means of a 0° optic with a 18 cm rigid endoscope followed by a maxillary antrostomy to gain access to the floor of the orbit. Then with a 45° optic the bony medial part of lamina papyracea and the floor of the orbit are identified and removed. After careful dissection from the overlying bone, the periorbita is sharply opened with sickle knife and endoscopic microscissors. Then the tumor become visible and is removed after dissection from the periorbital fat.

For intraconal lesions located inferiorly and medially, the dissection corridor is between the medial and inferior rectus muscles. They are identified and



isolated with vessel loop as they insert on the globe and then retracted. Once the intraconal corridor is developed, the tumor is identified and removed with limited bipolar cautery and extensive sharp dissection.

### Combined approaches

Campbell *et al.*<sup>[7]</sup> described a combined transcaruncular and a transnasal endoscopic cryo-assisted approach to remove a cavernous hemangioma. The Cryo-probe allowed freezing both at the tumor capsule surface and within the stroma, thus facilitating the endoscopic removal of the fluid-filled lesion.

Tsirbas *et al.*<sup>[22]</sup> performed, through an inferior transconjunctival orbitotomy, an orbital floor dissection subperiosteal to the posterior orbit to identify the anterior limit of the cavernous hemangioma. Then, by means of a transantral endoscopic approach, they removed the posterior orbital floor and gained the posterior orbital periosteum overlying the lesion, which was incised.

### Transorbital endoscopic approach

A transconjunctival transorbital endoscopic approach was recently described by Rivkin *et al.*<sup>[10]</sup> for the resection of a pleomorphic adenoma located in the posterior lateral orbit. The authors performed a lateral conjunctival incision, posterior to the lateral rectus muscle. No bone removal, craniotomy, or skin incision were required. Then, under the vision of a 0° endoscope, they made an incision in the periosteum and the tumor was delivered into the periosteal pocket.

The endoscope is a useful adjunct also for the treatment of selected orbital roof lesions, such as cholesterol granulomas, orbital dermoids and Langerhans cell histiocytosis involving the anterior portion of the orbital roof. In these cases bone removal is often needed for adequate visualization behind the superior orbital rim<sup>[23]</sup>.

Table 1 summarizes the hallmarks of aforementioned surgical approaches.

## DISCUSSION

Posterior orbit harbors pivotal neurovascular structures like the optic nerve, the ophthalmic artery and vein, and the ocular muscles and their nerves all crowded in a very narrow cone-shaped surgical field<sup>[22]</sup>. Thus the surgical approach planning is extremely challenging.

The location of the lesion inside the orbit is the most relevant parameter to consider in choosing the surgical approach. The approach is also decided on the basis of the extension and the type of the tumor<sup>[20]</sup>. The experience of the surgeon especially in endoscopic sinus surgery has also a role in the surgical planning process.

Traditional external orbital and cranial approaches involve risks associated with their invasiveness because they implies manipulation of delicate orbital structures

like extraocular muscles, which in some case need to be deinserted, with subsequent impact on extraocular mobility<sup>[9]</sup>.

Moreover lateral orbitotomy and supraorbital approach carry the disadvantage of postoperative scar. At the other hand the transconjunctival approach, which implies incision of the conjunctiva inferiorly along the corneal edge, without bone removal or skin incision, carry the disadvantage of a limited view; thus it is not suitable for large lesions.

The pterional approach offers an optimal view of the posterior orbit and of the upper part of the medial orbit thus providing good control of the optic canal, the superior orbital fissure and the anterior temporal fossa<sup>[24]</sup>. Extensive tissue manipulation connected with this approach can lead to the risk of injuring the frontal lobe along with intraorbital bleeding, cerebral edema and seizure.

The endonasal microscopic approach to the retrobulbar region described by Mir-Salim *et al.*<sup>[8]</sup> provides a limited view compared with the narrowness and deepness of the surgical field.

Different transnasal endoscopic approaches have been reported on for the treatment of orbital tumors, especially for intraconal lesions located inferiorly and medially to the optic nerve and the extraconal lesions adjacent to the paranasal sinuses.

A safe resection of orbital tumors through an endonasal endoscopic approach requires the respect of some key anatomic principles<sup>[9]</sup>. First, it is critical to avoid crossing the optic nerve. Thus, tumors that are localized to the superior/lateral orbit are contraindicated for an endonasal approach. Second, entering through the lamina papyracea below the level of the ethmoidal foramina allows sparing of the ethmoidal arteries thus reducing the risk of retrobulbar hemorrhage and vision disturbances. Finally, the dissection should occur between muscle groups rather than through individual muscles for preservation of function.

The avoidance of muscle detachment and the shortness of the surgical intraorbital trajectory makes endoscopic approach less invasive, thus minimizing tissue damage<sup>[25,26]</sup>. Endoscopic approach carries also the advantage to decrease the recovery time and to improve the cosmetic outcome not requiring skin incisions<sup>[26]</sup>.

Orbital surgery carries the risk of damaging the intraorbital structures because of a local increase of intraorbital pressure. In transnasal procedures, the removal of the lamina papyracea allows partial displacement of orbital content, otherwise collapsed in a not distensible space<sup>[25]</sup>.

The endoscopic surgery also carries some risks and distinct disadvantages. The first one is the lack of three-dimensional vision. However, moving the endoscope actively, thereby providing some sense of depth, can simulate a three-dimensional perception.

**Table 1** Hallmarks of the surgical approaches to the orbit

Approach	Ref.	Location	Size	Contraindication	Advantages	Disadvantages
Lateral orbitotomy	Arai <i>et al</i> <sup>[14]</sup> Carta <i>et al</i> <sup>[11]</sup>	Lateral, dorsal and basal to the ON	All	Medial location	Good view	Cosmetic scar
Transconjunctival	Cheng <i>et al</i> <sup>[16]</sup>	Basal and medial intra-extraconal tumors	Small	Medium size and large tumors	Minimally invasive	Limited view
Supraorbital	Maus <i>et al</i> <sup>[19]</sup>	Superior, lateral and medial	All	Basal location	Good view	Cosmetic scar
Pterional	Schick <i>et al</i> <sup>[24]</sup>	Superior and medial	All	Basal location	Good view	Invasive
Contralateral pterional	Hassler <i>et al</i> <sup>[20]</sup>	Superior and medial	All	Basal location	Good view	Invasive
Endonasal microsurgical	Mir-Salim <i>et al</i> <sup>[6]</sup>	Intraconal lesions	All	Lateral location	Three-dimensional view	Long approach distance and limited view
Endonasal endoscopic	Castelnuovo <i>et al</i> <sup>[25]</sup>	Inferior and medial to the ON, paranasal sinuses	Medium	Lateral location	Minimally invasive, better cosmetic outcome, short recovery time	Two visual dimensions, Small operative field
Combined	Transcaruncular and transnasal endoscopic cryo-assisted	Orbital apex	All	Solid consistency	To ablate vascular tumors	Cosmetic scar
	Inferior transconjunct, orbitotomy and transantral endoscopic	Posterior orbit, orbital apex	All	Medial location	Improved visualization and limited manipulation within the orbit	Cosmetic scar
Transorbital endoscopic	Rivkin <i>et al</i> <sup>[10]</sup>	Posterior lateral	All	Medial location	Decreased surgical morbidity, improved cosmesis	Two-dimensional view, learning curve

ON: Optic nerve.

Furthermore, the endoscopic sinus surgery allows a limited degree of space for instruments. Considering that the hemostasis could be difficult and risky in a small operative field, the endoscopic transnasal approach should be restricted mostly to benign tumors or inflammatory processes and not be used for highly vascularized tumors<sup>[27]</sup>.

Muscatello *et al*<sup>[21]</sup> noted that the consistency of cavernous hemangioma is ideal for the transnasal approach, because these lesion maintain their shape and can be easily manipulated without excessive risk of rupture. Moreover, unlike cerebral ones, which are not encapsulated, orbital cavernous hemangiomas are well encapsulated, probably by a specific reaction of the orbital fatty tissue<sup>[28]</sup> and the extracapsular dissection can be easily performed<sup>[23]</sup>. Furthermore, they consist of endothelium-lined dilated spaces with a low blood flow; this condition allows the surgeon to manipulate and to remove the lesion piecemeal when this procedure is necessary.

While endoscopic endonasal surgery is limited to intraconal lesions located inferiorly and medially to the optic nerve and to extraconal ones adjacent to the paranasal sinuses, tumors located in the lateral portion of the orbit can alternatively be managed using a

transconjunctival transorbital endoscopic approach, as recently described by Rivkin *et al*<sup>[10]</sup>. Comparing with external approaches, this technique proved to share the same advantages of the endonasal corridor such as decreased morbidity and post-operative pain, reduced hospitalization and improved cosmesis. Similarly, the technique has the limit of a two-dimensional view and the learning curve that may be required for surgeons less familiar with this surgery. Larger tumors or masses with bone involvement may still require standard open approaches.

## CONCLUSION

Endoscopic endonasal approach allows a useful and safe route to reach and manage orbital lesions located medially to the optic nerve. More traditional surgical approaches are still widely preferred but imply major surgical morbidity and invasiveness, so they can be avoided whenever an endoscopic endonasal approach should be performed. A multidisciplinary team with expertise in endoscopic techniques is mandatory.

## REFERENCES

- 1 Boari N, Gagliardi F, Castellazzi P, Mortini P. Surgical treatment

- of orbital cavernomas: clinical and functional outcome in a series of 20 patients. *Acta Neurochir (Wien)* 2011; **153**: 491-498 [PMID: 20872258 DOI: 10.1007/s00701-010-0808-1]
- 2 **Norris JL**, Cleasby GW. Endoscopic orbital surgery. *Am J Ophthalmol* 1981; **91**: 249-252 [PMID: 7468741]
- 3 **Norris JL**, Stewart WB. Bimanual endoscopic orbital biopsy. An emerging technique. *Ophthalmology* 1985; **92**: 34-38 [PMID: 3974993]
- 4 **Kasperbauer JL**, Hinkley L. Endoscopic orbital decompression for Graves' ophthalmopathy. *Am J Rhinol* 2005; **19**: 603-606 [PMID: 16402649]
- 5 **Pham AM**, Strong EB. Endoscopic management of facial fractures. *Curr Opin Otolaryngol Head Neck Surg* 2006; **14**: 234-241 [PMID: 16832179 DOI: 10.1097/01.moo.0000233593.84175.6e]
- 6 **Kuppersmith RB**, Alford EL, Patrinely JR, Lee AG, Parke RB, Holds JB. Combined transconjunctival/intranasal endoscopic approach to the optic canal in traumatic optic neuropathy. *Laryngoscope* 1997; **107**: 311-315 [PMID: 9121304 DOI: 10.1097/00005537-199703000-00006]
- 7 **Campbell PG**, Yadla S, Rosen M, Bilyk JR, Murchison AP, Evans JJ. Endoscopic transnasal cryo-assisted removal of an orbital cavernous hemangioma: a technical note. *Minim Invasive Neurosurg* 2011; **54**: 41-43 [PMID: 21509724 DOI: 10.1055/s-0030-1270465]
- 8 **Mir-Salim PA**, Berghaus A. [Endonasal, microsurgical approach to the retrobulbar region exemplified by intraconal hemangioma]. *HNO* 1999; **47**: 192-195 [PMID: 10231704]
- 9 **McKinney KA**, Snyderman CH, Carrau RL, Germanwala AV, Prevedello DM, Steffen ST, Gardner P, Kassam AB, Wheless SA, Zano AM. Seeing the light: endoscopic endonasal intraconal orbital tumor surgery. *Otolaryngol Head Neck Surg* 2010; **143**: 699-701 [PMID: 20974343 DOI: 10.1016/j.otohns.2010.07.010]
- 10 **Rivkin MA**, Turtz AR, Morgenstern KE. Transorbital endoscopic removal of posterior lateral orbital mass. *Laryngoscope* 2013; **123**: 3001-3004 [PMID: 23712481 DOI: 10.1002/lary.24228]
- 11 **Carta F**, Siccardi D, Cossu M, Viola C, Maiello M. Removal of tumours of the orbital apex via a postero-lateral orbitotomy. *J Neurosurg Sci* 1998; **42**: 185-188 [PMID: 10404745]
- 12 **Goldberg RA**, Shorr N, Arnold AC, Garcia GH. Deep transorbital approach to the apex and cavernous sinus. *Ophthal Plast Reconstr Surg* 1998; **14**: 336-341 [PMID: 9783284 DOI: 10.1097/00002341-199809000-00006]
- 13 **Shields JA**, Shields CL. Vascular and hemorrhagic lesions. In: Shields JA, Shields CL, editors. Atlas of orbital tumors. New York: Lippincott Williams and Wilkins, 1999: 45-73
- 14 **Arai H**, Sato K, Katsuta T, Rhoton AL. Lateral approach to intraorbital lesions: anatomic and surgical considerations. *Neurosurgery* 1996; **39**: 1157-162; discussion 1157-162; [PMID: 8938770 DOI: 10.1097/00006123-199612000-00018]
- 15 **Cockerham KP**, Bejjani GK, Kennerdell JS, Maroon JC. Surgery for orbital tumors. Part II: transorbital approaches. *Neurosurg Focus* 2001; **10**: E3 [PMID: 16724826]
- 16 **Cheng JW**, Wei RL, Cai JP, Li Y. Transconjunctival orbitotomy for orbital cavernous hemangiomas. *Can J Ophthalmol* 2008; **43**: 234-238 [PMID: 18347630 DOI: 10.3129/j08-005]
- 17 **Gdal-On M**, Gelfand YA. Surgical outcome of transconjunctival cryosurgical extraction of orbital cavernous hemangioma. *Ophthalmic Surg Lasers* 1998; **29**: 969-973 [PMID: 9854706]
- 18 **Geyer O**, Godel V, Lazar M. Transconjunctival approach for intraorbital tumors. *Arch Ophthalmol* 1988; **106**: 14-15 [PMID: 3337692 DOI: 10.1001/archophth.1988.01060130016006]
- 19 **Maus M**, Goldman HW. Removal of orbital apex hemangioma using new transorbital craniotomy through suprabrow approach. *Ophthal Plast Reconstr Surg* 1999; **15**: 166-170 [PMID: 10355834 DOI: 10.1097/00002341-199905000-00005]
- 20 **Hassler WE**, Meyer B, Rohde V, Unsöld R. Pterional approach to the contralateral orbit. *Neurosurgery* 1994; **34**: 552-554; discussion 554 [PMID: 8190236 DOI: 10.1227/00006123-199403000-00028]
- 21 **Muscatello L**, Seccia V, Caniglia M, Sellari-Franceschini S, Lenzi R. Transnasal endoscopic surgery for selected orbital cavernous hemangiomas: our preliminary experience. *Head Neck* 2013; **35**: E218-E220 [PMID: 22715119]
- 22 **Tsibras A**, Burt BO, Mancini R, Wormald PJ. Endoscopic surgery of the orbital apex. *Operative Techniques in Otolaryngology* 2008; **19**: 167-171 [DOI: 10.1016/j.otot.2008.09.003]
- 23 **Prabhakaran VC**, Selva D. Orbital endoscopic surgery. *Indian J Ophthalmol* 2008; **56**: 5-8 [PMID: 18158397 DOI: 10.4103/0301-4738.37587]
- 24 **Schick U**, Dott U, Hassler W. Surgical treatment of orbital cavernomas. *Surg Neurol* 2003; **60**: 234-244; discussion 244 [PMID: 12922043 DOI: 10.1016/S0090-3019(03)00136-8]
- 25 **Castellnuovo P**, Dallan I, Locatelli D, Battaglia P, Farneti P, Tomazic PV, Seccia V, Karligiotis A, Pasquini E, Stammberger H. Endoscopic transnasal intraorbital surgery: our experience with 16 cases. *Eur Arch Otorhinolaryngol* 2012; **269**: 1929-1935 [PMID: 22237761 DOI: 10.1007/s00405-011-1917-z]
- 26 **Murchison AP**, Rosen MR, Evans JJ, Bilyk JR. Endoscopic approach to the orbital apex and periorbital skull base. *Laryngoscope* 2011; **121**: 463-467 [PMID: 21344420 DOI: 10.1002/lary.21357]
- 27 **Yoshimura K**, Kubo S, Yoneda H, Hasegawa H, Tominaga S, Yoshimine T. Removal of a cavernous hemangioma in the orbital apex via the endoscopic transnasal approach: a case report. *Minim Invasive Neurosurg* 2010; **53**: 77-79 [PMID: 20533139]
- 28 **Hejazi N**, Hassler W, Offner F, Schuster A. Cavernous malformations of the orbit: a distinct entity? A review of own experiences. *Neurosurg Rev* 2007; **30**: 50-54; discussion 54-55 [PMID: 17089180 DOI: 10.1007/s10143-006-0055-3]

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## Clinical and diagnostic aspects of gluten related disorders

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**Author contributions:** Masi C analyzed the literature about non-celiac-gluten sensitivity and gluten ataxia revising the other sections; Guidetti E gathered the data about celiac disease revising the other sections; Negrini G analyzed trends in gluten related disorders in general with particular detail to non-celiac gluten sensitivity and revised celiac disease section; Paterini P analyzed current laboratory techniques used for the differential diagnosis of gluten-related diseases revising all of the sections; Tovoli F wrote the draft as a whole armonizing the various sections and updating bibliography; Bolondi L revised the final draft; all of the authors have seen and approve the final version of this paper.

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grains are widely consumed; in particular, wheat is one of the world's primary sources of food, providing up to 50% of the caloric intake in both industrialized and developing countries. Until two decades ago, celiac disease (CD) and other gluten-related disorders were believed to be exceedingly rare outside of Europe and were relatively ignored by health professionals and the global media. In recent years, however, the discovery of important diagnostic and pathogenic milestones led CD from obscurity to global prominence. In addition, interestingly, people feeding themselves with gluten-free products greatly outnumber patients affected by CD, fuelling a global consumption of gluten-free foods with approximately \$2.5 billion in United States sales each year. The acknowledgment of other medical conditions related to gluten that has arisen as health problems, providing a wide spectrum of gluten-related disorders. In February 2011, a new nomenclature for gluten-related disorders was created at a consensus conference in London. In this review, we analyse innovations in the field of research that emerged after the creation of the new classification, with particular attention to the new European Society for Paediatric Gastroenterology, Hepatology and Nutrition guidelines for CD and the most recent research about non-celiac gluten sensitivity.

**Key words:** Celiac disease; Wheat allergy; Gluten sensitivity; Non-celiac gluten sensitivity; Gluten-free diet; Gluten; Anti-gliadin antibodies

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**Core tip:** In recent years, there has been a widespread diffusion of gluten-associated symptoms. Current reactions to gluten include, but are not restricted to, celiac disease. This review analyses this interesting epidemiological worldwide phenomenon by discussing the spectrum of gluten-related disorders and focusing on their clinical features and diagnostic criteria. In particular, this paper will cover the most important news from European Society for Paediatric Gastroenterology,

### Abstract

Gluten is one of the most abundant and widely distributed components of food in many areas. It can be included in wheat, barley, rye, and grains such as oats, barley, spelt, kamut, and triticale. Gluten-containing



## Hepatology and Nutrition guidelines for celiac disease and the state of the art of non-celiac gluten sensitivity.

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## INTRODUCTION

In recent years, the prevalence of a wide spectrum of gluten-related disorders (GRDs) has increased. This can be attributed to changes in global dietary habits; many countries are experiencing a progressive westernization of diet as well as worldwide diffusion of the Mediterranean diet, which is based on a large number of foods that incorporate gluten (including wheat)<sup>[1]</sup>. In particular, consumption of wheat is progressively replacing consumption of rice in many countries in North Africa, the Middle East, and Asia<sup>[2]</sup>. Corn is still the most consumed cereal in the United States; however, a trend toward an increase in consumption of wheat is clearly evident, with an average of \$132.50 spent on wheat products per person<sup>[3]</sup>.

In addition, current wheat varieties have a higher content in gluten compared to the past due to changes directed by both technology and nutritional reasons.

Types of wheat cultivated for thousands of years, such as *Triticum monococcum* and *Triticum dicoccum*, contained smaller quantities of the highly toxic peptide 33-mer gliadin<sup>[4]</sup>.

The toxic effects of gluten are mediated in humans primarily by immunologic reactions; however, the absence of proper adaptation of gastrointestinal reactions can also play a role<sup>[2]</sup>. The mechanization of agriculture and the increasing use of industrial pesticides have encouraged the development of new types of wheat with a higher content of toxic peptides of gluten, constituting a further element in the increasing prevalence of GRDs<sup>[1]</sup>. Furthermore, bread and bakery products currently contain a higher portion of gluten than in the past because of the reduced time of dough fermentation<sup>[5]</sup>.

Diagnostic tools for GRDs have progressively improved over time<sup>[6,7]</sup>. In the 1980s, classification of GRDs was very simple, because celiac disease (CD) and dermatitis herpetiformis (DH) were the only known diseases with a well-documented role of gluten in their pathogenesis. More recently, gluten and other proteins have been recognized as a possible cause of wheat allergy (WA). In addition, more patients with intestinal and extraintestinal symptoms related to ingestion of gluten but without evidence of CD or WA have been identified as potentially affected by non-celiac

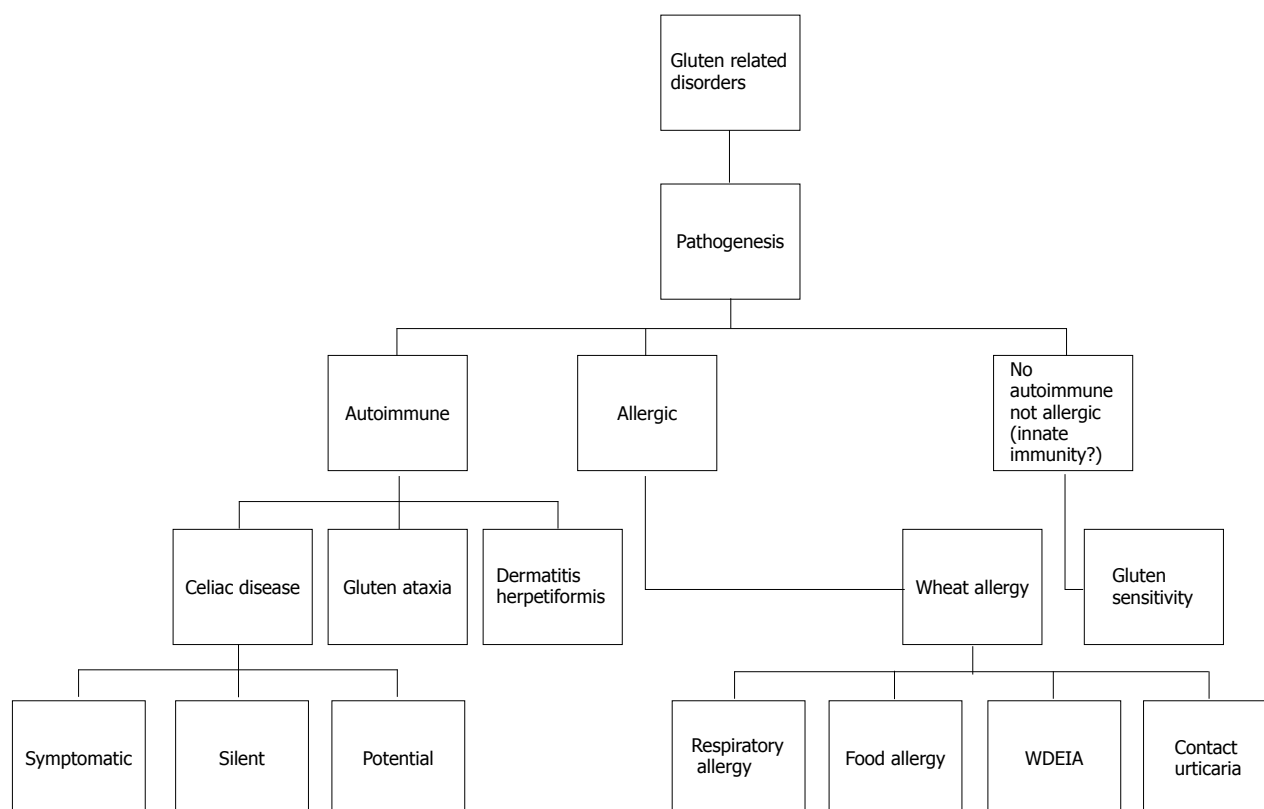
gluten sensitivity (NCGS), a disorder acknowledged by the scientific community only in recent times<sup>[8]</sup>. Increasing complexity in the nomenclature and clinical presentation of GRDs has led to the development of a consensus document by a panel of 15 experts on new classification of five heterogeneous GRDs: CD, NCGS, WA, DH, and gluten ataxia (GA)<sup>[9]</sup> (Figure 1). Each GRD exhibits a unique pathophysiological response to ingestion of gluten, although there can show considerable overlap in the clinical presentation.

The increased prevalence and complexity of GRDs has inevitably sparked growing interest in gluten-free diets (GFDs) in both scientific and non-scientific communities. Although a GFD represents the recommended treatment of GRDs and is believed by many people to be an overall healthier regimen, this is not always the case. People following a GFD may not meet their nutritional requirements because gluten-free foods may not have the same dietary supplementation as gluten-containing foods<sup>[10]</sup>. However, a GFD can contribute to meet daily nutritional requirements provided that patients will use a healthful balance of protein, vegetables, fruit, and ancient grains<sup>[8]</sup>. Even if gluten may not be essential for a healthy diet, an unrequired GFD can be expensive. Furthermore, recognizing gluten-free products can be difficult and time consuming<sup>[11,12]</sup>.

In 2010, the gluten-free food market was worth an estimated \$2.6 billion in the United States, showing a steady increase since 2008. This upward trend is expected to continue in the next years<sup>[9,13]</sup>. The growing gluten-free market may now be between 15% and 20% of the United States population<sup>[14]</sup>. The increased awareness and knowledge of CD explains only a small fraction of the development of the GFD market, which is probably sustained also by people with different GRDs, such as NCGS and WA. The remaining section of the market includes people who embark on a GFD as occasional users who do not have a medical necessity but believe that popular cereals are unhealthy because of their composition<sup>[15]</sup>. Consequently, accurate diagnostic criteria for a GFD are needed to distinguish people with a medical condition from those who simply prefer to avoid gluten, leading to different nutrition and follow-up strategies.

## CD

CD is an immune-mediated reaction to gluten; it is characterized by an inappropriate T cell-mediated immune response that causes inflammatory injury to the small intestine in genetically predisposed subjects carrying the HLA-DQ2 and/or -DQ8 haplotypes<sup>[9]</sup>. CD represents a unique model of autoimmune disease because relevant information is known, including the genetic basis (HLA haplotypes) and the triggering environmental factor (gluten)<sup>[9]</sup>. The disease epidemiology is also well known, with the worldwide prevalence estimated to be 0.6%



Proposed new nomenclature and classification of gluten-related disorders.  
Sapone *et al.* *BMC Medicine* 2012; 10: 13 DOI: 10.1186/1741-7015-10-12

**Figure 1** New nomenclature and proposed classification of gluten related disorders according to the the II Consensus Conference on gluten related disorders held in London in February 2011.

to 1% of the general population<sup>[16-19]</sup>. For each person diagnosed with CD, there are at least another five or six people who have not yet been identified, most of whom are adults without gastrointestinal symptoms (representing the so-called celiac iceberg)<sup>[20]</sup>.

CD is a diagnostic challenge for the clinician because it may develop at any age, even in elderly people, and because of its polymorphic clinical presentation. The clinical spectrum of CD includes symptomatic cases with either intestinal or extraintestinal features as well as silent forms revealed only by serological screening. Intestinal manifestations of CD include diarrhoea, weight loss, abdominal distention, and constipation. Extraintestinal symptoms reflect the systemic nature of the disease and include chronic fatigue, anaemia, reduced bone mineral density, aphthous stomatitis, high aminotransferase levels, joint/muscle pain, and spontaneous abortions, epilepsy, peripheral neuropathy<sup>[21]</sup>.

In 2012, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Working Group decided to revise its classification of CD. In particular, the distinction between classic and atypical CD based on symptoms was removed, because atypical signs and symptoms (e.g., anaemia, reduced bone density, neuropathy) can be substantially more common than classic symptoms (e.g., abdominal pain, chronic diarrhoea)<sup>[22]</sup>.

In such a complex clinical picture, case-finding strategies have to be carefully planned. The most relevant scientific organizations in this field, including ESPGHAN, North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition, and American College of Gastroenterology, have identified groups of patients at particularly high risk that should be investigated for CD. Their recommendations are reported in Table 1<sup>[22-24]</sup>. Diagnostic algorithms for CD consist of initial screening serological tests followed by a confirmatory small intestinal biopsy.

Measurement of serum immunoglobulin (Ig) A anti-tissue transglutaminase antibodies (tTG) has the best diagnostic sensitivity for CD<sup>[21,25,26]</sup>. Measurement of IgA anti-endomysial antibodies (EMA) is nearly 100% specific for CD, but it is also expensive and operator dependent and therefore is better used as a second-line test<sup>[22]</sup>. Antibodies to deamidated gliadin peptides (DGP) of the IgG class have been shown to be particularly useful in patients with IgA deficiency and children younger than three years of age<sup>[26-28]</sup>. Even with the most recent advancements in CD serology, it has been reported that up to 2% of patients with CD do not have any circulating markers of gluten sensitivity, defining a condition of seronegative CD<sup>[6]</sup>.

Since the advent of the Crosby-Kugler capsule, which enabled tissue sampling and histological examination of

**Table 1** Populations at risk for celiac disease, in which investigations for celiac disease are indicated, according to the most recent guidelines proposed by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition, North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and American College of Gastroenterology

ESPGHAN	NASPGHAN	ACG
Test children/adolescent with: Chronic or intermittent diarrhoea, growth failure, weight loss Chronic abdominal pain, cramping or distension, nausea or vomiting, chronic constipation Short stature, delayed puberty dermatitis herpetiformis-type rash unexplained abnormal liver biochemistry Iron-deficiency anaemia repetitive fractures/osteopenia/osteoporosis chronic fatigue, ameorrhoea, recurrent aphthous stomatitis First- degree family members Type 1-diabetes mellitus Other associated conditions <sup>1</sup>	Test children/adolescent with: diarrhea and failure to thrive abdominal pain, anorexia, constipation, vomiting short stature, delayed puberty dermatitis herpetiformis  Iron-deficient anaemia resistant to oral iron osteoporosis Dental enamel hypoplasia of permanent teeth First-degree family members Type 1-diabetes mellitus Other associated conditions <sup>1</sup>	Test patients with: chronic diarrhea with weight loss post-prandial abdominal pain, bloating  other symptoms/signs suspect for CD  unexplained abnormal liver biochemistry other laboratory signs suspect for CD  First- degree family members Type 1-diabetes mellitus

<sup>1</sup>Down syndrome, autoimmune thyroid disease, Turner syndrome, Williams syndrome, IgA deficiency. ESPGHAN guidelines also consider autoimmune liver diseases. CD: Celiac disease; ESPGHAN: European Society for Pediatric Gastroenterology, Hepatology, and Nutrition; NASPGHAN: North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition; ACG: American College of Gastroenterology.

intestinal tissue, small intestinal biopsy has been required to confirm the diagnosis in patients with suspected CD and is considered the gold standard for CD<sup>[20]</sup>. Interestingly, the most recent guidelines from ESPGHAN propose that it may be possible to avoid intestinal biopsy in children who meet the following criteria: (1) symptoms consistent with CD; (2) serum IgA anti-tTG levels > 10 times the upper limit of normal (confirmed with positive anti-EMA in a different blood sample); and (3) positive HLA-DQ2<sup>[22]</sup>. It remains to be determined whether this new clinical/laboratory standard functions as well as the gold standard of serology plus biopsy, whether different cutoff values for serology kits should be used, and whether including HLA typing is necessary<sup>[29]</sup>. Nevertheless, with this notable exception, biopsy is still a required and essential element for the diagnosis of CD according to all of the guidelines<sup>[22-24]</sup>.

Histology alone, however, is not specific for the diagnosis of CD, particularly if villous atrophy is absent. For instance, an increase in the number of lymphocytes in the intestinal epithelium can be found in a number of different conditions, including small bowel bacterial overgrowth, drug-associated enteropathy, infectious enteritis (e.g., giardiasis and Whipple disease), Crohn's disease, autoimmune enteropathy, and enteropathy associated with acquired immunodeficiency syndrome<sup>[24]</sup>. Genetic studies have identified HLA-DQ2 and -DQ8 as the major determinants of susceptibility to CD. Because these haplotypes are common in the general population, determination of HLA is better suited for ruling out the presence of CD in suspicious cases<sup>[22]</sup>.

In light of the aforementioned complexities, the final diagnosis of CD must be based on a comprehensive evaluation of clinical, serological, histological, and, when indicated, genetic elements. This consideration is known as the "4 out of 5 rule".

As such, four of these five criteria should be satisfied for diagnosing CD: (1) presence of symptoms associated

with CD; (2) presence of CD-associated autoantibodies (i.e., tTG, IgA EMA); (3) presence of HLA-DQ2 or -DQ8 alleles; (4) duodenal biopsy demonstrating blunting or absence of villi (Marsh III) with > 25 lymphocytes/100 enterocytes (with cluster of differentiation 3+ staining); and (5) melioration of symptoms after a GFD<sup>[30]</sup>.

As we improve our understanding of the pathogenesis of CD, the interplay of genetic, epigenetic, and environmental factors may need to be considered as part of the diagnostic process<sup>[29]</sup>. In this regard, in recent years, more studies have investigated possible novel biomarkers of CD. Intriguing studies have identified CD4<sup>+</sup> gluten-DQ2 tetramers in the peripheral blood of patients with CD after a short gluten challenge<sup>[31]</sup>. These cells were not present in controls or in patients with CD while on a GFD. More recently, Galatola *et al.*<sup>[32]</sup> reported that a small gene expression panel from peripheral blood monocytes could discriminate between patients with active CD and healthy controls. The intestinal microbiome is another novel field of interest in CD, potentially leading to further understanding of its pathogenic mechanism and to the discovery of new markers of disease. An overall lack of Bifidobacteria and high abundance of Firmicutes were found in children with genetic susceptibility for CD who had early exposure to gluten in a study by Sellitto *et al.*<sup>[33]</sup>, which examined a small cohort of at-risk infants and controls up to 24 mo of age. The investigators suggest that there may be predictive ability in measuring such biomarkers. Viitasalo *et al.*<sup>[34]</sup> found significant evidence of high levels of antibodies to ASCA (anti-Saccharomyces cerevisiae antibodies), OmpW (Bacteroides caccae TonB-linked outer membrane protein), and I2 (Pseudomonas fluorescens-associated sequence) in patients with early-stage lesions. Because antibody titres decreased after introduction of a GFD, the investigators proposed that Bacteroides and Pseudomonas species may play a part in the pathogenesis of CD. These results should encourage studies of novel biomarkers as we advance toward the possibility of a biopsy-free diagnosis of CD

because they may add further security to the diagnosis of CD.

## DH

DH is a skin disease characterized by a blistering rash and pathognomonic cutaneous IgA deposits<sup>[35]</sup>. Dühring's original description also included patients with different conditions, such as erythema multiforme and pemphigus.

Differently from other GRDs, the prevalence of DH is higher in men than in women (1.5 to 1.9:1).

DH shares an high prevalence of HLA alleles DQ2 (90%) and DQ8 (5%) with CD<sup>[36]</sup>. Even if a skin rash or other dermatologic manifestations can be common in untreated CD<sup>[37]</sup>, DH presents with unique characteristics. The usual clinical presentation of DH consists of diffuse, symmetrical, grouped polymorphic lesions consisting of erythema, urticarial plaques, papules, herpetiform vesiculae, and blisters followed by erosions, excoriations, and hyperpigmentation<sup>[38,39]</sup>. DH most frequently involves the extensor surfaces of the elbows (90%), knees (30%), shoulders, sacral region, buttocks, and face. Itching of variable intensity, scratching, and burning sensation immediately preceding the development of lesions are common.

Gastrointestinal symptoms in patients with DH are uncommon (affecting approximately 10% of patients) and usually mild<sup>[38,39]</sup>. However, 65% to 75% of patients show a celiac-type intestinal atrophy<sup>[9]</sup>. Even in patients with apparently normal biopsy specimens, an increased number of intraepithelial lymphocytes can be found consistently with a gluten sensitization<sup>[9]</sup>. Although often asymptomatic in adults, small bowel involvement in patients with DH can be associated with abdominal pain, diarrhoea, iron deficiency, and reduced growth rates in children<sup>[38,39]</sup>.

Celiac-type serological markers (anti-tTG, anti-EMA, anti-DGP antibodies) can be typically found in DH patients. In a recent study, Borroni *et al.*<sup>[40]</sup> identified IgA anti-epidermal transglutaminase autoantibodies as a promising marker for the serological diagnosis of DH<sup>[40]</sup>.

The revelation of IgA by immunofluorescence staining on biopsy specimens of uninvolved skin analysed is another key diagnostic element<sup>[41]</sup>. Differently from other autoimmune disorders such as pemphigus, which can display homogeneous linear IgA deposits, IgA are detected as granular or fibrillar deposits in the dermal papillae in DH. Sometimes IgA can be found in linear granular deposits along the basement membrane as well<sup>[41]</sup>.

Final diagnosis of Dühring disease can be made according to the histopathologic findings on skin specimen and presence of celiac related antibodies<sup>[41]</sup>. Once diagnosis have been formulated duodenal biopsies can be avoided, as it is commonly recognized that DH represents the skin counterpart of CD<sup>[41]</sup>.

## GA

GA is perhaps the most dramatic representation of neurological involvement in the setting of an immune response to gluten-containing foods. It has been defined as an otherwise unexplained sporadic ataxia with presence of serological antibodies consistent with a condition of sensitization to gluten<sup>[42]</sup>. Its predominant clinical manifestations include dysarthria, dysphonia, pyramidal signs, nystagmus and other ocular signs of cerebellar dysfunction (in up to 80% of cases), progressive ataxia of gait associated with myoclonus, palatal tremor, or opsoclonus<sup>[43]</sup>. Only very few subjects with GA experience any gastrointestinal symptoms; however, almost one-third of these patients have histological evidence for small bowel villous atrophy on biopsy specimens<sup>[9]</sup>. The precise pathogenic mechanism of GA is not clear, but a number of factors probably play different roles. Vitamin deficiency (in particular, vitamin E and vitamin B1) secondary to malabsorption in the small intestine may play a role; however, there is also evidence for a toxic and immune-mediated response to gluten<sup>[44]</sup>. Different studies suggest that some subjects with GA have both anti-gliadin antibodies and antibodies reacting with Purkinje cells. The latter marker is not present in patients with other causes of ataxia, resulting a pathognomonic feature of GA<sup>[44]</sup>.

It is believed that the Purkinje cells of the cerebellum share epitopes with gliadin proteins and, as further evidence for the role of the immune system, intravenous immune globulin therapy has been reported to improve ataxia<sup>[44]</sup>. Recently, tTG6 has been identified in specimens of nervous tissue from patients with GA<sup>[45]</sup>. The molecular structure of tTG6 is similar to the tTG2 molecule involved in the development of CD and to the tTG3 molecule linked to DH<sup>[45]</sup>. Interestingly, anti-tTG6 deposits of the IgA class have also been found around brain vessels of patients with GA<sup>[45]</sup> and circulating anti-tTG6 antibodies appear to be a specific and sensitive marker for GA<sup>[46]</sup>. In light of these considerations, any subject with an history of progressive cerebellar ataxia of unknown origin should be regarded as possibly affected by GA, and tested for anti-gliadin antibodies (AGA), classical anti-tTG and possibly anti-tTG6 IgG and IgA antibodies<sup>[9]</sup>. Patients displaying positivity for any of these antibodies should be followed up for one year on a GFD. Stabilization or improvement of the ataxia associated with the negativity of antibodies should also be evaluated after the first year on a GFD<sup>[9]</sup>.

## WA

WA can be defined as an adverse reaction of the immune system to the proteins contained in wheat.

In the vast field of WA a further classification can be made, distinguishing: (1) a classical form of food allergy with involvement of gastrointestinal tract,



skin and possibly respiratory tract; (2) a typical form of inhalant allergy (baker's asthma and rhinitis); (3) wheat- dependent, exercise-induced anaphylaxis (WDEIA); and (4) more rarely, a form of contact urticaria. The role of IgE-mediated reactions in the pathogenesis of baker's asthma and rhinitis has been demonstrated as early as the beginning of the 20<sup>th</sup> century<sup>[47]</sup>.

Symptoms consisting with baker's asthma have been referred by 4.2% of bakery workers after a single year, increasing after 8.6% after the second working year<sup>[48]</sup>. Symptoms include rhinitis, skin itching/rash, ocular symptoms (including tearing, itching, and conjunctival injection), respiratory symptoms (including coughing, wheezing, shortness of breath, and sputum production), and "grain fever"<sup>[49-51]</sup>.

Alfa-amylase inhibitors are the considered the most prominent allergen involved in the generation of symptoms, but different wheat proteins can play a role<sup>[52]</sup>.

Alimentary WA, in its turn, is a less common disease, however in its most severe forms it can lead to serious reactions, including anaphylaxis and death<sup>[53]</sup>.

In contrast to CD, symptoms of WA are typical for an IgE-mediated allergy, including itching and swelling in the mouth, nose, eyes, and throat; skin rash or swelling; wheezing in the respiratory tract; gastrointestinal symptoms such as cramps, bloating, and diarrhoea; and life-threatening anaphylaxis<sup>[54]</sup>. Differently from CD, WA does not cause permanent gastrointestinal damage<sup>[54]</sup>.

WDEIA is a separate form of WA that is induced by physical exercise after ingestion of wheat. A specific type of grain protein,  $\omega$ 5-gliadin, acts as a trigger factor. Exercise within three hours of wheat consumption can induce an adverse reaction in susceptible people. In some cases, this can also occur when wheat is consumed directly after exercise<sup>[9,53]</sup>. Patients with WDEIA can refer different symptoms, ranging from urticaria to anaphylaxis and other severe reactions<sup>[55]</sup>. Although the mechanism of physical exercise-induced anaphylaxis is indistinguishable, an immediate-type hypersensitivity to water/salt-insoluble fraction of gluten has been considered to underlie this disease<sup>[53]</sup>.

Skin prick tests and IgE assays are considered first-level studies for diagnosis of WA<sup>[9]</sup>. However, interpretation of these tests can be difficult because different confounding factors should be considered. First, a number of commercial kits for skin prick tests lack in sensitivity because they do not include allergens deriving from the insoluble gliadin fraction. Second, cross reactions with grass pollens may be present (especially in adults), resulting in lower specificity<sup>[9]</sup>. Testing prick by prick with raw material potentially overcomes these problems; nevertheless a definite diagnosis still requires an oral food challenge as a final test in a number of cases<sup>[9]</sup>.

## NCGS

NCGS is the "youngest" member of the GRD family and is characterized by intestinal and extraintestinal symptoms that occur after the ingestion of gluten-containing food in subjects in whom CD and WA have been ruled out. Rapid disappearance of symptoms with a GFD and recurrence a few hours/days after gluten reintroduction are also characteristic of this condition<sup>[56]</sup>.

The existence of NCGS was firstly postulated in 1980 by Cooper *et al*<sup>[57]</sup>, who described an evident amelioration of symptoms (bloating, diarrhoea and abdominal pain) in 6 out of 8 women after gluten withdrawal, in absence of the diagnostic criteria for CD.

After 20 years without further mention of this condition, Kaukinen *et al*<sup>[58]</sup> reported in 2000 that the majority of patients complaining gluten-related symptoms were non actually classifiable as being affected by CD or WA. Since they had a clear benefit from gluten withdrawal, their condition was called NCGS.

In the past 10 years, NCGS has received growing attention as patients have reported more severe nonspecific symptoms with intake of gluten by accident than seen with classic CD<sup>[59]</sup>. The recent Consensus Conference on GRD<sup>[9]</sup> is a clear sign of the scientific interest surrounding this clinical entity, even if little is still known about NCGS, especially when compared with current advancements in CD. For instance, reliable studies regarding the actual prevalence of this condition are still lacking. In current studies, prevalence of NCGS ranges from 0.63% in a primary care program<sup>[60]</sup> to 6% in a tertiary care centre<sup>[9]</sup>.

The pathogenesis of NCGS is another hot topic. Since it was first described, a link between gluten and symptoms was suggested in subjects with NCGS<sup>[57]</sup>. Indeed, gluten itself has opioid-like activity because gluten proteins can alter the intestinal transit time in healthy volunteers and its action is reverted by naloxone<sup>[61]</sup>. Furthermore, an experimental model with transgenic mice gliadin-sensitized demonstrated increased secretion of acetylcholine from the myenteric plexus, with consequential enhancement of muscle contractility and increase in epithelial secretion. Gluten withdrawal was able to revert these abnormalities<sup>[62]</sup>. However, recent studies suggested that gluten may not be the only triggers of NCGS, with different wheat proteins likely playing relevant roles in this condition. For example, some grains and cereals (such as wheat, rye, and barley) are known to be particularly rich in fermentable oligosaccharides, disaccharides, and monosaccharides and polyols (FODMAPs). In turn, FODMAPs are known to provoke gastrointestinal symptoms in patients with irritable bowel syndrome through mechanisms involving gut microbiota, gas production, and fermentation<sup>[63]</sup>. Similarly to irritable bowel syndrome, FODMAPs can possibly play a role

in generating both intestinal and extraintestinal manifestations in subjects with NCGS. Recent studies have shown that a diet low in FODMAPs results in improved symptoms in patients with self-reported gluten intolerance, supporting the hypothesis of a major role of FODMAPs compared to gluten<sup>[64,65]</sup>. Furthermore, wheat amylase and trypsin inhibitors, a complex of proteins in innate immunity, could contribute to the origination of symptoms in NCGS<sup>[7]</sup>.

Because it is unclear which component of wheat-based products is responsible for an individual's symptoms, it may be premature to assign all of the blame to gluten. From a clinical point of view, patients with NCGS may display great variability in gastrointestinal (bloating, abdominal pain, diarrhoea, nausea, aerophagia, aphthous stomatitis, constipation) and extraintestinal symptoms (lack of well-being, tiredness, headache, anxiety, foggy mind, numbness, joint or muscle pain, skin rash, anaemia, dermatitis)<sup>[66]</sup>.

Interestingly, mood disorders in NCGS may recognize similar pathophysiological mechanisms to other neurological manifestations observed in gluten-related disorders such as GA, as reiterated toxic insults might an impaired immunological tolerance (*i.e.*, the so-called "toxicant induced loss of tolerance")<sup>[67]</sup>.

Some papers also suggested a relationship between NCGS and neuropsychiatric diseases, with a particular regard to autism and schizophrenia, however the responsibility of gluten in conditions affecting the nervous system remains hot topic requiring additional studies<sup>[68]</sup>.

Other important clinical aspects of NCGS are its frequent occurrence in first-degree relatives of patients with CD and a straightforward prevalence in female subjects (6:1)<sup>[66]</sup>. Heterogeneity in clinical presentation with identification of various subgroups of patients has been noticed by some investigators<sup>[65,69]</sup>, possibly reflecting different pathogenic roles for the various proteins and carbohydrates contained in wheat and other gluten-rich cereals. Consequently, it has been speculated that NCGS only provides the best current description of a heterogeneous group of conditions with the common feature of improvement in symptoms on withdrawal of gluten<sup>[70]</sup>. At this time, it appears that NCGS is not associated with malabsorption or nutritional deficiencies or with any increased risk of autoimmune disorders or intestinal malignancy<sup>[24]</sup>. Therefore, differently from subjects affected by CD, patients with NCGS should not fear contaminations due to inadvertently introduced traces of gluten.

As noted in the preceding text, before diagnosing NCGS other conditions such as WA or CD should be excluded with appropriate tests during a gluten-containing diet.

WA should be excluded by testing for serum IgE antibodies to gluten and wheat fractions and by skin prick tests, while CD must be ruled out by the negativity of celiac-specific antibodies, *i.e.*, IgA tTG,

IgA EMA, and IgG DGP. A duodenal biopsy is also highly recommended because of the possibility of seronegative CD, occurring in 1%-2% of all patients with CD<sup>[6]</sup>.

HLA-DQ2 and -DQ8 apotypes are present in 50% of subjects with NCGS, representing a low prevalence compared with CD (95%), only slightly higher than in the general population (30%)<sup>[9]</sup>. Genetic studies investigating non-HLA regions are still lacking and, in general, the immunogenetics of NCGS are still non-existent<sup>[71]</sup>.

Once identified negative criteria for the diagnosis of NCGS, the double-blind, placebo-controlled challenge (DBPCC) was proposed as a first positive diagnostic criterion. DBPCC trials have been highly as a confirmatory test for NCGS because of a possible placebo effect generated by gluten withdrawal<sup>[9]</sup>. However, a DBPCC is time-consuming and requires a close follow-up of patients and thus is currently used only in research<sup>[70]</sup>.

The identification of other positive criteria and diagnostic markers is of great interest in NCGS. Recently, an elegant retrospective study by Kabbani *et al.*<sup>[72]</sup> analysed 238 patients with symptoms responsive to GFD without prior diagnosis or exclusion of CD, demonstrating that patients with CD and patients with NCGS may have different clinical presentations. In particular, patients with NCGS were less likely to have malabsorptive symptoms, nutrient deficiency, and a personal history of autoimmune diseases, which is consistent with previous reports<sup>[24]</sup>. Consequently, the investigators concluded that patients who improve with a GFD but have negative findings on serology, lack of malabsorptive symptoms, and absence of risk factors for CD are likely to have NCGS and may not need to routinely undergo diagnostic endoscopy<sup>[72]</sup>. Nonetheless, these interesting findings need to be validated by future prospective studies.

In regard to serological markers, it was recently shown that 56% of patients with NCGS have circulating IgG AGA antibodies<sup>[73]</sup>, which is consistent with the results from other investigators<sup>[69]</sup>. Interestingly, after starting a GFD, almost all of the patients with NCGS had normalization of AGA IgG levels, whereas these antibodies were still present in 40% of patients with CD after gluten withdrawal<sup>[74]</sup>. Strict compliance with and a good response to a GFD, with significant improvement in symptoms, were significantly related to the disappearance of AGA IgG in patients with NCGS<sup>[74]</sup>. Still, AGA IgG cannot be considered a reliable biomarker of NCGS because it can be detected in multiple different disorders, including autoimmune diseases, as well as in healthy subjects. As the cost of DNA sequencing is spectacularly reducing, it could be interesting for the clinicians to characterize the different subgroups of patients with this condition<sup>[71]</sup>. Consequently, further research on biomarkers of NCGS

is strongly encouraged.

## CONCLUSION

Based on the data presented in this report, it is clear that clinical manifestations of GRDs cover a wide variety of medical specialties, ranging from gastroenterology to allergology and from neurology to dermatology. Within the large family of GRDs, two opposite trends seem evident. First, we have a great deal of information about the clinical presentation, pathogenesis, and diagnostic markers of some diseases (with CD as a prototypic example). Second, there are some more recently accepted conditions, such as NCGS, with many clinical and diagnostic aspects still to be investigated. Consequently, different priorities are required for different situations. In regard to CD, identification of new early microbial or non-microbial markers represents a promising field of interest, eventually leading to an early and non-invasive diagnosis in the future. In the case of NCGS, first we have to understand whether it constitutes a singular entity or only provides the best description of a heterogeneous group of conditions attributable to different wheat-related food constituents. Only studies with more homogeneous groups of patients will be able to provide relevant information on this apparently frequent but still elusive condition.

## REFERENCES

- Volta U, Caio G, Tovoli F, De Giorgio R. Non-celiac gluten sensitivity: questions still to be answered despite increasing awareness. *Cell Mol Immunol* 2013; **10**: 383-392 [PMID: 23934026 DOI: 10.1038/cmi.2013.28]
- Catassi C, Cobellis G. Coeliac disease epidemiology is alive and kicking, especially in the developing world. *Dig Liver Dis* 2007; **39**: 908-910 [PMID: 17720636 DOI: 10.1016/j.dld.2007.07.159]
- Wheat's role in the US diet. US Department of Agriculture. 2013. Available from: URL: <http://www.ers.usda.gov/homepage> [on the Internet]
- Molberg O, Uhlen AK, Jensen T, Flaete NS, Fleckenstein B, Arentz-Hansen H, Raki M, Lundin KE, Sollid LM. Mapping of gluten T-cell epitopes in the bread wheat ancestors: implications for celiac disease. *Gastroenterology* 2005; **128**: 393-401 [PMID: 15685550 DOI: 10.1053/j.gastro.2004.11.003]
- Gobbetti M, Giuseppe Rizzello C, Di Cagno R, De Angelis M. Sourdough lactobacilli and celiac disease. *Food Microbiol* 2007; **24**: 187-196 [PMID: 17008163]
- Volta U, Villanacci V. Celiac disease: diagnostic criteria in progress. *Cell Mol Immunol* 2011; **8**: 96-102 [PMID: 21278763 DOI: 10.1038/cmi.2010.64]
- Inomata N. Wheat allergy. *Curr Opin Allergy Clin Immunol* 2009; **9**: 238-243 [PMID: 19318930 DOI: 10.1097/ACI.0b013e32832aa5bc]
- Leonard MM, Vasagar B. US perspective on gluten-related diseases. *Clin Exp Gastroenterol* 2014; **7**: 25-37 [PMID: 24493932 DOI: 10.2147/CEG.S54567]
- Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M, Kaukinen K, Rostami K, Sanders DS, Schumann M, Ullrich R, Villalta D, Volta U, Catassi C, Fasano A. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Med* 2012; **10**: 13 [PMID: 22313950 DOI: 10.1186/1741-7015-10-13]
- Hallert C, Grant C, Grehn S, Grännö C, Hultén S, Midhagen G, Ström M, Svensson H, Valdimarsson T. Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years. *Aliment Pharmacol Ther* 2002; **16**: 1333-1339 [PMID: 12144584]
- Verrill L, Zhang Y, Kane R. Food label usage and reported difficulty with following a gluten-free diet among individuals in the USA with coeliac disease and those with noncoeliac gluten sensitivity. *J Hum Nutr Diet* 2013; **26**: 479-487 [PMID: 23347179 DOI: 10.1111/jhn.12032]
- Lee AR, Ng DL, Zivin J, Green PH. Economic burden of a gluten-free diet. *J Hum Nutr Diet* 2007; **20**: 423-430 [PMID: 17845376]
- Lundin KE, Alaedini A. Non-celiac gluten sensitivity. *Gastrointest Endosc Clin N Am* 2012; **22**: 723-734 [PMID: 23083989 DOI: 10.1016/j.giec.2012.07.006]
- Biesiekierski JR, Muir JG, Gibson PR. Is gluten a cause of gastrointestinal symptoms in people without celiac disease? *Curr Allergy Asthma Rep* 2013; **13**: 631-638 [PMID: 24026574 DOI: 10.1007/s11882-013-0386-4]
- Lundin KE. Non-celiac gluten sensitivity - why worry? *BMC Med* 2014; **12**: 86 [PMID: 24885490 DOI: 10.1186/1741-7015-12-86]
- Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Green PH, Guandalini S, Hill ID, Pietzak M, Ventura A, Thorpe M, Kryszak D, Fornaroli F, Wasserman SS, Murray JA, Horvath K. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch Intern Med* 2003; **163**: 286-292 [PMID: 12578508]
- Mustalahti K, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S, Murray L, Metzger MH, Gasparin M, Bravi E, Mäki M. The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. *Ann Med* 2010; **42**: 587-595 [PMID: 21070098 DOI: 10.3109/07853890.2010.505931]
- Gandolfi L, Pratesi R, Cordoba JC, Taul PL, Gasparin M, Catassi C. Prevalence of celiac disease among blood donors in Brazil. *Am J Gastroenterol* 2000; **95**: 689-692 [PMID: 10710058]
- Sood A, Midha V, Sood N, Kaushal V, Puri H. Increasing incidence of celiac disease in India. *Am J Gastroenterol* 2001; **96**: 2804-2805 [PMID: 11569725]
- Bizzaro N, Tozzoli R, Villalta D, Fabris M, Tonutti E. Cutting-edge issues in celiac disease and in gluten intolerance. *Clin Rev Allergy Immunol* 2012; **42**: 279-287 [PMID: 21181303 DOI: 10.1007/s12016-010-8223-1]
- Fasano A, Catassi C. Clinical practice. Celiac disease. *N Engl J Med* 2012; **367**: 2419-2426 [PMID: 23252527 DOI: 10.1056/NEJMc1113994]
- Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, Troncone R, Giersiepen K, Branski D, Catassi C, Leigeman M, Mäki M, Ribes-Koninckx C, Ventura A, Zimmer KP. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 2012; **54**: 136-160 [PMID: 22197856 DOI: 10.1097/MPG.0b013e31821a23d0]
- Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, Hoffenberg EJ, Horvath K, Murray JA, Pivor M, Seidman EG. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2005; **40**: 1-19 [PMID: 15625418]
- Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA. ACG clinical guidelines: diagnosis and management of celiac disease. *Am J Gastroenterol* 2013; **108**: 656-676; quiz 677 [PMID: 23609613 DOI: 10.1038/ajg.2013.79]
- Kaukinen K, Lindfors K, Collin P, Koskinen O, Mäki M. Coeliac disease--a diagnostic and therapeutic challenge. *Clin Chem Lab Med* 2010; **48**: 1205-1216 [PMID: 20578966 DOI: 10.1515/CCLM.2010.241]
- Leffler DA, Schuppan D. Update on serologic testing in celiac disease. *Am J Gastroenterol* 2010; **105**: 2520-2524 [PMID: 21131921 DOI: 10.1038/ajg.2010.276]
- Villalta D, Tonutti E, Prause C, Koletzko S, Uhlig HH, Vermeersch P, Bossuyt X, Stern M, Laass MW, Ellis JH, Ciclitira PJ, Richter T,



- Daehnrich C, Schlumberger W, Mothes T. IgG antibodies against deamidated gliadin peptides for diagnosis of celiac disease in patients with IgA deficiency. *Clin Chem* 2010; **56**: 464-468 [PMID: 20022984 DOI: 10.1373/clinchem.2009.128132]
- 28 Amarri S, Alvisi P, De Giorgio R, Gelli MC, Cicola R, Tovoli F, Sassatelli R, Caio G, Volta U. Antibodies to deamidated gliadin peptides: an accurate predictor of coeliac disease in infancy. *J Clin Immunol* 2013; **33**: 1027-1030 [PMID: 23558824 DOI: 10.1007/s10875-013-9888-z]
- 29 Zevit N, Shamir R. Diagnosis of celiac disease: where are we heading after the ESPGHAN 2012 guidelines? *J Pediatr Gastroenterol Nutr* 2014; **59** Suppl 1: S13-S15 [PMID: 24979193 DOI: 10.1097/01.mpg.0000450396.76521.b0]
- 30 Catassi C, Fasano A. Celiac disease diagnosis: simple rules are better than complicated algorithms. *Am J Med* 2010; **123**: 691-693 [PMID: 20670718 DOI: 10.1016/j.amjmed.2010.02.019]
- 31 Råki M, Fallang LE, Brottveit M, Bergseng E, Quarsten H, Lundin KE, Sollid LM. Tetramer visualization of gut-homing gluten-specific T cells in the peripheral blood of celiac disease patients. *Proc Natl Acad Sci USA* 2007; **104**: 2831-2836 [PMID: 17307878]
- 32 Galatola M, Izzo V, Cielo D, Morelli M, Gambino G, Zanzi D, Strisciuglio C, Sperandeo MP, Greco L, Auricchio R. Gene expression profile of peripheral blood monocytes: a step towards the molecular diagnosis of celiac disease? *PLoS One* 2013; **8**: e74747 [PMID: 24069342 DOI: 10.1371/journal.pone.0074747]
- 33 Sellitto M, Bai G, Serena G, Fricke WF, Sturgeon C, Gajer P, White JR, Koenig SS, Sakamoto J, Boothe D, Gicquelais R, Kryszak D, Puppa E, Catassi C, Ravel J, Fasano A. Proof of concept of microbiome-metabolome analysis and delayed gluten exposure on celiac disease autoimmunity in genetically at-risk infants. *PLoS One* 2012; **7**: e33387 [PMID: 22432018 DOI: 10.1371/journal.pone.0033387]
- 34 Viitasalo L, Niemi L, Ashorn M, Ashorn S, Braun J, Huhtala H, Collin P, Mäki M, Kaukinen K, Kurppa K, Iltaanen S. Early microbial markers of celiac disease. *J Clin Gastroenterol* 2014; **48**: 620-624 [PMID: 24518796 DOI: 10.1097/MCG.0000000000000089]
- 35 Salmi TT, Hervonen K, Kautiainen H, Collin P, Reunala T. Prevalence and incidence of dermatitis herpetiformis: a 40-year prospective study from Finland. *Br J Dermatol* 2011; **165**: 354-359 [PMID: 21517799 DOI: 10.1111/j.1365-2133.2011.10385.x]
- 36 Holmes G, Catassi C, Fasano A. Dermatitis Herpetiformis in Celiac disease. Oxford: Health Press, 2009: 83-90
- 37 Bonciolini V, Antiga E, Fabbri P, Caproni M. Skin manifestations of celiac disease: not always dermatitis herpetiformis. *Int J Dermatol* 2014; **53**: e352-e353 [PMID: 24601945 DOI: 10.1111/ijd.12350]
- 38 Nicolas ME, Krause PK, Gibson LE, Murray JA. Dermatitis herpetiformis. *Int J Dermatol* 2003; **42**: 588-600 [PMID: 12890100]
- 39 Fry L. Dermatitis herpetiformis: problems, progress and prospects. *Eur J Dermatol* 2002; **12**: 523-531 [PMID: 12459520]
- 40 Borroni G, Biagi F, Ciocca O, Vassallo C, Carugno A, Cananzi R, Campanella J, Bianchi PI, Brazzelli V, Corazza GR. IgA anti-epidermal transglutaminase autoantibodies: a sensible and sensitive marker for diagnosis of dermatitis herpetiformis in adult patients. *J Eur Acad Dermatol Venereol* 2013; **27**: 836-841 [PMID: 22672004 DOI: 10.1111/j.1468-3083.2012.04586.x]
- 41 Caproni M, Antiga E, Melani L, Fabbri P. Guidelines for the diagnosis and treatment of dermatitis herpetiformis. *J Eur Acad Dermatol Venereol* 2009; **23**: 633-638 [PMID: 19470076 DOI: 10.1111/j.1468-3083.2009.03188.x]
- 42 Hadjivassiliou M, Grünewald RA, Chattopadhyay AK, Davies-Jones GA, Gibson A, Jarratt JA, Kandler RH, Lobo A, Powell T, Smith CM. Clinical, radiological, neurophysiological, and neuropathological characteristics of gluten ataxia. *Lancet* 1998; **352**: 1582-1585 [PMID: 9843103]
- 43 Hadjivassiliou M, Grünewald R, Sharrack B, Sanders D, Lobo A, Williamson C, Woodroffe N, Wood N, Davies-Jones A. Gluten ataxia in perspective: epidemiology, genetic susceptibility and clinical characteristics. *Brain* 2003; **126**: 685-691 [PMID: 12566288]
- 44 Hadjivassiliou M, Boscolo S, Davies-Jones GA, Grünewald RA, Not T, Sanders DS, Simpson JE, Tongiorgi E, Williamson CA, Woodroffe NM. The humoral response in the pathogenesis of gluten ataxia. *Neurology* 2002; **58**: 1221-1226 [PMID: 11971090]
- 45 Stamnaes J, Dorum S, Fleckenstein B, Aeschlimann D, Sollid LM. Gluten T cell epitope targeting by TG3 and TG6; implications for dermatitis herpetiformis and gluten ataxia. *Amino Acids* 2010; **39**: 1183-1191 [PMID: 20300788 DOI: 10.1007/s00726-010-0554-y]
- 46 Hadjivassiliou M, Aeschlimann P, Sanders DS, Mäki M, Kaukinen K, Grünewald RA, Bandmann O, Woodroffe N, Haddock G, Aeschlimann DP. Transglutaminase 6 antibodies in the diagnosis of gluten ataxia. *Neurology* 2013; **80**: 1740-1745 [PMID: 23576621 DOI: 10.1212/WNL.0b013e3182919070]
- 47 Houba R, Doekes G, Heederik D. Occupational respiratory allergy in bakery workers: a review of the literature. *Am J Ind Med* 1998; **34**: 529-546 [PMID: 9816411]
- 48 Walusiak J, Hanke W, Górski P, Palczyński C. Respiratory allergy in apprentice bakers: do occupational allergies follow the allergic march? *Allergy* 2004; **59**: 442-450 [PMID: 15005769]
- 49 Matsumura Y, Niitsuma T, Ito H. A study of factors contributing to bakers' allergy symptoms. *Aerugi* 1994; **43**: 625-633 [PMID: 7518230]
- 50 Merget R, Sander I, van Kampen V, Beckmann U, Heinze E, Raulf-Heimsoth M, Bruening T. Allergic asthma after flour inhalation in subjects without occupational exposure to flours: an experimental pilot study. *Int Arch Occup Environ Health* 2011; **84**: 753-760 [PMID: 21279645 DOI: 10.1007/s00420-011-0617-8]
- 51 Baldo BA, Krilis S, Wrigley CW. Hypersensitivity to inhaled flour allergens. Comparison between cereals. *Allergy* 1980; **35**: 45-56 [PMID: 6154431]
- 52 Tatham AS, Shewry PR. Allergens to wheat and related cereals. *Clin Exp Allergy* 2008; **38**: 1712-1726 [PMID: 18823308 DOI: 10.1111/j.1365-2222.2008.03101.x]
- 53 Pasha I, Saeed F, Sultan MT, Batool R, Aziz M, Ahmed W. Wheat allergy & intolerance; recent updates and perspectives. *Crit Rev Food Sci Nutr* 2013 Sep 2; Epub ahead of print [PMID: 24915366]
- 54 Pietzak M. Celiac disease, wheat allergy, and gluten sensitivity: when gluten free is not a fad. *JPEN J Parenter Enteral Nutr* 2012; **36**: 68S-75S [PMID: 22237879]
- 55 Palosuo K, Varjonen E, Kekki OM, Klemola T, Kalkkinen N, Alenius H, Reunala T. Wheat omega-5 gliadin is a major allergen in children with immediate allergy to ingested wheat. *J Allergy Clin Immunol* 2001; **108**: 634-638 [PMID: 11590393]
- 56 Troncone R, Jabri B. Coeliac disease and gluten sensitivity. *J Intern Med* 2011; **269**: 582-590 [PMID: 21481018 DOI: 10.1111/j.1365-2796.2011.02385.x]
- 57 Cooper BT, Holmes GK, Ferguson R, Thompson RA, Allan RN, Cooke WT. Gluten-sensitive diarrhea without evidence of celiac disease. *Gastroenterology* 1980; **79**: 801-806 [PMID: 7419003]
- 58 Kaukinen K, Turjanmaa K, Mäki M, Partanen J, Venäläinen R, Reunala T, Collin P. Intolerance to cereals is not specific for coeliac disease. *Scand J Gastroenterol* 2000; **35**: 942-946 [PMID: 11063153]
- 59 Mulder CJ, van Wanrooij RL, Bakker SF, Wierdsma N, Bouma G. Gluten-free diet in gluten-related disorders. *Dig Dis* 2013; **31**: 57-62 [PMID: 23797124 DOI: 10.1159/000347180]
- 60 DiGiacomo DV, Tennyson CA, Green PH, Demmer RT. Prevalence of gluten-free diet adherence among individuals without celiac disease in the USA: results from the Continuous National Health and Nutrition Examination Survey 2009-2010. *Scand J Gastroenterol* 2013; **48**: 921-925 [PMID: 23834276 DOI: 10.3109/00365521.2013.809598]
- 61 Corazza GR, Frazzoni M, Stocchi A, Prati C, Sarchielli P, Capelli M. Alimentary exorphin actions on motility and hormonal secretion of gastrointestinal tract. In Fraioli F, Isidori A, Mazzetti M (ed.). Opioid Peptides in the Periphery. Amsterdam: Elsevier Sciences Publisher, 1984: 243-247
- 62 Verdu EF, Huang X, Natividad J, Lu J, Blennerhassett PA, David CS, McKay DM, Murray JA. Gliadin-dependent neuromuscular and epithelial secretory responses in gluten-sensitive HLA-DQ8 transgenic mice. *Am J Physiol Gastrointest Liver Physiol* 2008;



- 294: G217-G225 [PMID: 18006603]
- 63 **Halmos EP**, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology* 2014; **146**: 67-75.e5 [PMID: 24076059 DOI: 10.1053/j.gastro.2013.09.046]
  - 64 **Biesiekierski JR**, Peters SL, Newnham ED, Rosella O, Muir JG, Gibson PR. No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. *Gastroenterology* 2013; **145**: 320-8.e1-320-8.e3 [PMID: 23648697 DOI: 10.1053/j.gastro.2013.04.051]
  - 65 **Zanini B**, Baschè R, Ferraresi A, Lanzarotto F, Marullo M, Ricci C, Lanzini A. PTH-111 “non Celiac Gluten Sensitivity” (ncgs) Is Uncommon In Patients Spontaneously Adhering To Gluten Free Diet (gfd), And Is Outnumbered By “fodmaps Sensitivity”. *Gut* 2014; **63**: A260 [DOI: 10.1136/gutjnl-2014-307263.557]
  - 66 **Volta U**, Bardella MT, Calabrò A, Troncone R, Corazza GR. An Italian prospective multicenter survey on patients suspected of having non-celiac gluten sensitivity. *BMC Med* 2014; **12**: 85 [PMID: 24885375 DOI: 10.1186/1741-7015-12-85]
  - 67 **Genuis SJ**, Lobo RA. Gluten sensitivity presenting as a neuropsychiatric disorder. *Gastroenterol Res Pract* 2014; **2014**: 293206 [PMID: 24693281 DOI: 10.1155/2014/293206]
  - 68 **Catassi C**, Bai JC, Bonaz B, Bouma G, Calabrò A, Carroccio A, Castillejo G, Ciacci C, Cristofori F, Dolinsek J, Francavilla R, Elli L, Green P, Holtmeier W, Koehler P, Koletzko S, Meinhold C, Sanders D, Schumann M, Schuppan D, Ullrich R, Vécsei A, Volta U, Zavallos V, Sapone A, Fasano A. Non-Celiac Gluten sensitivity: the new frontier of gluten related disorders. *Nutrients* 2013; **5**: 3839-3853 [PMID: 24077239 DOI: 10.3390/nu5103839]
  - 69 **Carroccio A**, Mansueto P, Iacono G, Soresi M, D’Alcamo A, Cavataio F, Brusca I, Florena AM, Ambrosiano G, Seidita A, Pirrone G, Rini GB. Non-celiac wheat sensitivity diagnosed by double-blind placebo-controlled challenge: exploring a new clinical entity. *Am J Gastroenterol* 2012; **107**: 1898-1906; quiz 1907 [PMID: 22825366 DOI: 10.1038/ajg.2012.236]
  - 70 **Mooney PD**, Aziz I, Sanders DS. Non-celiac gluten sensitivity: clinical relevance and recommendations for future research. *Neurogastroenterol Motil* 2013; **25**: 864-871 [PMID: 23937528 DOI: 10.1111/nmo.12216]
  - 71 **Pena AS**. Immunogenetics of non celiac gluten sensitivity. *Gastroenterol Hepatol Bed Bench* 2014; **7**: 1-5 [PMID: 25436091]
  - 72 **Kabbani TA**, Vanga RR, Leffler DA, Villafuerte-Galvez J, Pallav K, Hansen J, Mukherjee R, Dennis M, Kelly CP. Celiac disease or non-celiac gluten sensitivity? An approach to clinical differential diagnosis. *Am J Gastroenterol* 2014; **109**: 741-746; quiz 747 [PMID: 24619056 DOI: 10.1038/ajg.2014.41]
  - 73 **Volta U**, Tovoli F, Cicola R, Parisi C, Fabbri A, Piscaglia M, Fiorini E, Caio G. Serological tests in gluten sensitivity (nonceliac gluten intolerance). *J Clin Gastroenterol* 2012; **46**: 680-685 [PMID: 22138844 DOI: 10.1097/MCG.0b013e3182372541]
  - 74 **Caio G**, Volta U, Tovoli F, De Giorgio R. Effect of gluten free diet on immune response to gliadin in patients with non-celiac gluten sensitivity. *BMC Gastroenterol* 2014; **14**: 26 [PMID: 24524388 DOI: 10.1186/1471-230X-14-26]

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## Asthma and metabolic syndrome: Current knowledge and future perspectives

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### Abstract

Asthma and obesity are epidemiologically linked; however, similar relationships are also observed with other markers of the metabolic syndrome, such as insulin resistance and dyslipidemia, which cannot be accounted for by increased body mass alone. Obesity appears to be a predisposing factor for the asthma onset, both in adults and in children. In addition, obesity could make asthma more difficult to control and to treat. Although obesity may predispose to increased Th2 inflammation or tendency to atopy, other

mechanisms need to be considered, such as those mediated by hyperglycaemia, hyperinsulinemia and dyslipidemia in the context of metabolic syndrome. The mechanisms underlying the association between asthma and metabolic syndrome are yet to be determined. In the past, these two conditions were believed to occur in the same individual without any pathogenetic link. However, the improvement in asthma symptoms following weight reduction indicates a causal relationship. The interplay between these two diseases is probably due to a bidirectional interaction. The purpose of this review is to describe the current knowledge about the possible link between metabolic syndrome and asthma, and explore potential application for future studies and strategic approaches.

**Key words:** Asthma; Metabolic syndrome; Obesity; Hyperinsulinemia; Dyslipidemia

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**Core tip:** Asthma is a complex syndrome that encompasses multiple phenotypes. The relationship with obesity has been addressed in the past; however, the underlying mechanism of such a relationship seems to be more complex, and not explained by the body weight alone. The metabolic syndrome carries a condition of systemic inflammation that could potentially explain the influence on asthma onset and severity. This is a rather unexplored area that could potentially open new scenario in the diagnostic algorithm and in the strategic approach, with a more comprehensive assessment of the disease.

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## INTRODUCTION

Asthma is among the most common chronic diseases worldwide. The disease is poorly controlled despite available therapies in a large proportion of patients<sup>[1]</sup>, with long-term impairment and disability<sup>[2-4]</sup>. Among factors impairing the control of symptoms and the lack of response to treatment, obesity is to be taken into account, as stated by recent guidelines<sup>[5]</sup>.

It is well recognized that obesity and asthma are epidemiologically linked<sup>[6-9]</sup>. This relationship is also observed between asthma and other markers of the metabolic syndrome, such as insulin resistance and hypertension that cannot be accounted for by increased body mass alone<sup>[9-12]</sup>. The World Health Organization has reported that obesity has dramatically increased during the last few decades. In 2009-2010, more than one-third of United States adults (35.7%) were obese<sup>[13]</sup>. In this scenario, an estimated 300000 deaths per year are directly attributable to obesity, mainly due to heart diseases, diabetes, cancer, obstructive sleep apnea syndrome (OSAS), arthritis, and psychological disturbances, leading to the concept that obesity represents a risk factor for several pathologies in different clinical conditions<sup>[14]</sup>. In this regard, overweight and obesity have been demonstrated to be associated in a dose-dependent fashion with the risk of having asthma<sup>[15]</sup>, and obesity appears to be a predisposing factor for the asthma onset, both in adults and in children, as assessed by several cross-sectional studies<sup>[16]</sup>. In addition, obesity could make asthma more difficult to control and to treat; interestingly, weight-loss interventions in overweight severe asthmatic patients have shown substantial improvements in the clinical status, lung function, symptoms, and overall asthma control<sup>[8,17,18]</sup>. However, the mechanism linking obesity and asthma is still a controversial issue.

The obese-asthma phenotype is characterized by a paucity of airway inflammation. Although obesity may predispose to increased Th2 inflammation or tendency to atopy, other mechanisms that are independent of inflammatory infiltrates need to be considered, such as hyperglycaemia, hyperinsulinemia and dyslipidemia in the context of metabolic syndrome. Metabolic syndrome is defined as a syndrome that involves three of the following characteristics: dyslipidemia (high levels of apoB lipoproteins and triglycerides, and/or low high density lipoprotein cholesterol), an impaired fasting glucose metabolism, hypertension or central obesity<sup>[19-21]</sup>. Metabolic syndrome is directly involved in the increased prevalence of coronary heart disease, atherosclerotic diseases, and diabetes mellitus type 2<sup>[20-22]</sup>. Other metabolic abnormalities have been reported in patients with metabolic syndrome (chronic proinflammatory and prothrombotic states, liver disease and sleep apnea)<sup>[20-22]</sup>. In the literature, some authors consider that the aforementioned criterion is a combination of risk factors rather than a specific syndrome<sup>[23]</sup>. On the other hand, epidemiological data

reveals that there is a high prevalence of metabolic syndrome in both childhood and young adulthood, and pattern seems to be related to several inflammatory diseases including asthma<sup>[22]</sup>.

## EPIDEMIOLOGICAL LINK BETWEEN ASTHMA AND METABOLIC SYNDROME

In obese individuals, the risk for asthma in overweight and obese individuals is increased and does not differ with gender<sup>[24,25]</sup>. In a recent report, Dandona *et al.*<sup>[26]</sup> showed that in obese asthma patients, with or without type 2 diabetes, there is an increased expression of pro-inflammatory mediators. Following gastric bypass surgery and weight loss, the expression of the aforementioned mediators and plasma metabolites fall significantly suggesting that the pro-inflammatory effect of obesity can be downregulated upon adipose tissue reduction. Assad *et al.*<sup>[27]</sup> recently showed that BMI predicts asthma in women more than metabolic syndrome<sup>[28]</sup>, however, Agrawal *et al.*<sup>[29]</sup> suggested that calculation of parameters was conducted on entirely different scales, thereby limiting comparison of strength. In another study, Brumpton *et al.*<sup>[11]</sup> evaluated the associations of metabolic syndrome with the cumulative incidence of asthma in adults in 23245 individuals after an 11 years follow up (Nord-Trøndelag Health Study 1999-2008), showing that metabolic syndrome predisposes to. In a large mendelian randomization study, Granell *et al.*<sup>[30]</sup> recently found that higher BMI increases the risk of asthma in non-atopic (1.90, 95%CI: 1.19-3.03) and atopic children (1.37, 95%CI: 0.89-2.11).

## PATHOPHYSIOLOGICAL MECHANISMS

Obesity-associated asthma is characterized by the presence of neutrophilic airway inflammation, increased morbidity, and resistance to corticosteroids. The mechanisms underlying the relationship between metabolic syndrome and asthma are yet to be fully understood<sup>[31]</sup>. In the past, these two conditions were believed to occur in the same individual without any pathogenetic link. However, the improvement of asthma symptoms following weight reduction implies a causal relationship between obesity and asthma<sup>[32,33]</sup>. The interplay between these two diseases could be based on a bidirectional interaction. For example, obese asthmatics are at higher risk of metabolic syndrome as opposed to obese individuals who do not suffer from asthma, suggesting that asthma *per se* can increase the risk of developing metabolic syndrome<sup>[34]</sup>. Similarly, metabolic syndrome has been demonstrated to increase the severity of asthma<sup>[35,36]</sup>. Recently, changes in the expression of pro-inflammatory mediators such as leptin, IL-6, TNF- $\alpha$ , C-reactive protein and adiponectin have been demonstrated in obese asthmatics<sup>[37]</sup>, implying their potential role in the pathogenesis of

obesity-associated asthma. However, due to the paucity of available literature in this area, it appears difficult to draw definite conclusions until additional experimental and epidemiological data are collected.

A cross-sectional study published by Bruno *et al.*<sup>[38]</sup> recently analyzed the influence of BMI on asthma control in subjects with severe forms of the disease, demonstrating that the optimal state of asthma control is lower in obese than in normal weight and in overweight severe asthmatics and the number of asthma exacerbation episodes are significantly higher in obese than in normal or overweight severe asthmatics. These results may be explained with the inflammatory cascade that the adipose tissue generates. Indeed, the obese state is characterized by the so-called low-grade systemic inflammation<sup>[38]</sup>. Subcutaneous fat is the major source of fatty acids for the liver, and of free fatty acids in the circulating plasma<sup>[39,40]</sup>. Subcutaneous fat is related to insulin resistance and to visceral adipose tissue<sup>[39,40]</sup>. Abdominal subcutaneous fat from obese subjects has been reported to be an inflamed adipose state characterized by tissue macrophage accumulation. This pathologic tissue has been associated with impaired local vasodilatation, peripheral hyperinsulinemia, and insulin resistance<sup>[39-41]</sup>. Macrophage presence in the tissue is associated with an increase of plasma high-sensitivity C-reactive protein (hsCRP) levels and local amounts of TNF- $\alpha$ <sup>[39,40]</sup>. The precise mechanism of this event remains to be elucidated; however, adipokines have been proposed as important endocrine mediators since they are related to adipose tissue function and modulation. The following proteins are listed as adipokines, which are envisaged as markers of fat body mass and distribution, as well as tissue function: (1) leptin; (2) adiponectin; (3) ghrelin; (4) vaspin; (5) retinol binding protein 4; (6) apelin; (7) progranulin and MCP-1; (8) omentin; (9) resistin and chemerin; and (10) fetuin<sup>[42,43]</sup>. Adipose derived hormones may represent molecular links between asthma and inflammation. For example, adiponectin is known to exert anti-inflammatory effects, by inhibiting the eosinophil functions. Indeed, pre-treatment with adiponectin has been demonstrated to diminish the eotaxin-mediated chemotactic responses, by binding the adiponectin receptors AdipoR1 and AdipoR2 that are expressed in human eosinophils<sup>[44,45]</sup>. In addition, adiponectin has been shown to act as a protector to human bronchial epithelial cell that are involved in the pathogenesis of asthma<sup>[46]</sup>.

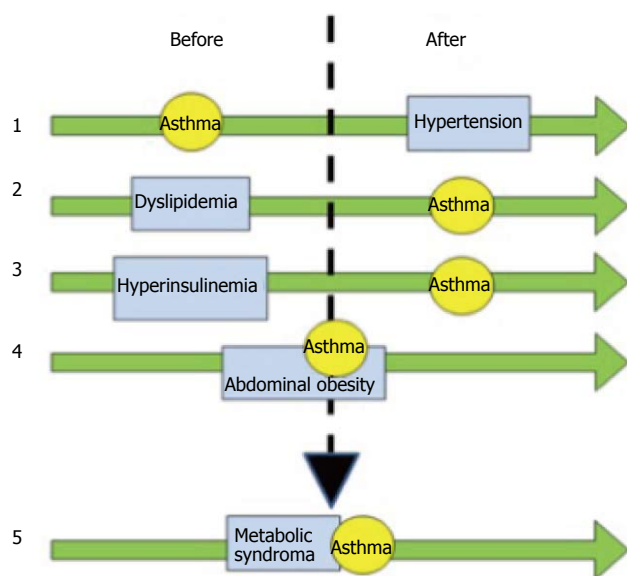
High serum levels of resistin have been recently documented in asthmatic children<sup>[47]</sup>. More important, an *in vitro* study showed that the resistin production strongly increases in obese patients with severe persistent asthma<sup>[48]</sup>, providing support to the notion that resistin can be depicted as a pro-inflammatory cytokine mainly in severely obese asthmatics. Conversely, high leptin levels are associated with

a more severe disease and this even in non-obese asthmatics<sup>[49,50]</sup>. Leptin can upregulate systemic inflammation and may lead to an impairment in lung function<sup>[51]</sup>. Increased expression and secretion of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-6, and IL-12 were detected when exposed to leptin<sup>[52]</sup>. Also, the systemic inflammation may contribute to drive insulin resistance, endothelial dysfunction and high blood pressure conditions. The results of a survey confirmed that leptin levels were highly associated with asthma especially in premenopausal women independent of BMI<sup>[51]</sup>. Guler *et al.*<sup>[53]</sup> also suggested that serum leptin concentrations were a predictive factor for asthma in boys, even after adjusting for obesity. Previously, leptin-mediated increased bronchial hyperactivity in obese mice models had been documented<sup>[32]</sup>.

The changes in the adipose tissue in metabolic syndrome favour the production of mediators that modulate the transcription factors. When they are activated by their ligands, they are able to control genes that are involved in intermediate metabolism<sup>[54]</sup>. In this regard, peroxisome proliferator-activated receptors (PPAR)-gamma agonists may attenuate the upper airway allergic inflammation by induction of T<sub>reg</sub> cells and inhibiting the proliferation of effector T cells<sup>[55]</sup>.

Diet-induced dyslipidemia may affect the trafficking of immune cells to the lung in diseases such as asthma<sup>[56]</sup>. In pulmonary physiology, circulating low density and high density lipoproteins (LDL and HDL) are both taken up by specific receptors, and consequently block local cholesterol biosynthesis<sup>[56]</sup>. Alveolar cholesterol homeostasis has been demonstrated to affect surfactant synthesis in normal lung physiology<sup>[56]</sup>. Conversely, HDL promotes surfactant production, and lung fibroblast growth. Adipose tissue reduction by diet or surgery, modulation of cholesterol, or glucose metabolism, has an important effect in asthmatic patients. The apolipoprotein E (ApoE)-low density lipoprotein receptor pathway appears to be involved in the pathogenesis of a murine model of allergic asthma<sup>[1]</sup>. However, the mechanism by which this protein modulates asthma pathogenesis has never been fully elucidated. ApoE has been hypothesized to as negatively modulate the degree of airway hyperresponsiveness<sup>[57]</sup>. Perhaps, this mechanism can also apply to humans. Low levels of serum HDL were found to be associated with an increased risk for asthma in adolescence<sup>[58]</sup>, and a recent analysis on 85555 adults demonstrated that high triglycerides and low HDL were associated with wheezing, supporting their role as markers of inflammation<sup>[59]</sup>. Recently, the association between LDL and asthma was investigated by Scichilone *et al.*<sup>[60]</sup>, who found that in mild asthmatics, the least pro-inflammatory LDL (LDL-1 and LDL-2) are lower than in healthy subjects, whereas the most pro-inflammatory (LDL-3 and LDL-4) are higher. In addition, the serum concentrations of LDL-3 (most pro-inflammatory) were





**Figure 1 Relationships between asthma and features of metabolic syndrome.** The different conditions are divided into "before" and "after" to explain which occurred earlier, implying a causal association. The green arrows describe the temporal evolution and the time when the diseases occurred. Arrow 1 sets asthma as a risk factor for systemic hypertension due to the chronic use of corticosteroids. Arrows 2 and 3 depict the role of dyslipidemia and hyperinsulinemia as risk factors for asthma, due to the abnormalities of the lipoprotein pattern and the influence on the M2 receptors, respectively. Arrow 4 shows the bidirectional association between asthma and obesity. Arrow 5 summarizes the influence of the above-described associations, showing the tight relationship between metabolic syndrome and asthma. Also see text for the explanation.

negatively associated with lung function, suggesting their contribution to the occurrence of the inflammatory changes of the airways<sup>[60]</sup>. Insulin excess can also directly alter lung cellular physiology and this would represent a fundamental common molecular link between asthma and metabolic syndrome<sup>[61]</sup>. There is substantial data that mechanistically links insulin and insulin like growth factor-1 to lung development and function. It is conceivable, although not proven, that hyperinsulinemia may lead to development of lung disease, particularly asthma<sup>[62]</sup>. Experimental studies that directly address this possibility are strongly advocated.

Recent observations seem to focus on the mitochondrial dysfunction as main mediator of the pathogenetic link between metabolic syndrome and asthma. Defective mitochondrial biogenesis in the adipose tissue is well documented in metabolic syndrome<sup>[63-66]</sup>. However, the involvement of mitochondria alterations among the risk factors of metabolic syndrome and asthma is unknown<sup>[67-72]</sup>.

Oxidative stress on both pulmonary and extra-pulmonary inflammation in obesity may play a major role<sup>[73,74]</sup>. Oxidative stress is characterized by increased reactive oxygen species (ROS), which induce functional changes of the airways. On this basis, increased oxidative stress may be recognized

as a potential mechanism by which obesity results in increased asthma severity. In this regard, the renin angiotensin aldosterone system, a potent inducer of oxidative stress, is often activated in patients with metabolic syndrome, and results in increased levels of angiotensin II. Angiotensin II seems to be able to determine bronchial hyperresponsiveness<sup>[75]</sup> and airway remodelling<sup>[76]</sup>; however, the mechanisms by which this occurs are not yet fully understood.

## CURRENT AND FUTURE DEVELOPMENTS

Figure 1 describes the temporal and causal relationships between asthma and features of metabolic syndrome. The role of lipoproteins in the pathogenesis of asthma pathogenesis supported the use of statins in asthmatic patients<sup>[77-83]</sup>; however, there are still some controversies<sup>[84-86]</sup>. Even though the aim of statin use in asthmatic patients is related to cholesterol metabolism, most of the reports have highlighted the anti inflammatory properties<sup>[77-79,86,87]</sup>. An *in vitro* study showed that lovastatin attenuates the differentiation and proliferation of asthmatic bronchial fibroblasts<sup>[85]</sup>, airway smooth muscle cells<sup>[86]</sup>. Both simvastatin and atorvastatin treatment reduce inflammatory cells in sputum<sup>[86]</sup>. The mevalonate-dependent and-independent pathways have been identified as potential opportunities for novel treatments with statins in asthma develop new treatments for asthma<sup>[78]</sup>. Even though statin therapy could be beneficial for a subgroup of asthmatic patients that are either overweight or obese, similar important advantages can be obtained by diet and exercise<sup>[20,26]</sup>. Biphosphonates could have a beneficial effect in asthmatic patients; alendronate has been shown to have a protective effect by decreasing eosinophil airway inflammation by chemokine secretion, eotaxin, and down-regulating cytokine secretion induced by Th2 and Th17 cells<sup>[88]</sup>. Retinoic acid<sup>[89]</sup>, retinoids<sup>[90]</sup> and fenretidine<sup>[91]</sup> appear to have a beneficial effect on the inflammatory asthmatic response by decreasing the inflammatory milieu. As a consequence, signal transduction pathways inhibition by these compounds could decrease the occurrence of bronchial constriction. Further studies are required to ascertain the possible beneficial effect of new therapeutic elements to control hypertension and endocrine disorders in asthmatic patients with metabolic syndrome. In patients with severe uncontrolled or non responding asthma<sup>[92,93]</sup>, biological therapies seem to be relevant. Interestingly, chemokines and chemokine receptors, CCR3 and CCR4, have been involved in adipose tissue mass increase and insulin resistance<sup>[94,95]</sup>. Thus, therapy involving chemokines or chemokine receptor inhibition could potentially provide beneficial effects on asthmatic patients with metabolic syndrome<sup>[96]</sup>. Finally, a therapeutic option in asthmatic patients with diabetes could be represented by thiazolidinediones, oral diabetes medications that selectively activate

PPAR receptor gamma, which have potent anti-inflammatory properties thus reducing the number of exacerbations<sup>[97,98]</sup>.

## CONCLUSION

The scope of the current review is to explore the possible link between metabolic syndrome and asthma. Early endocrine disturbances seem to predispose to severe or difficult to treat asthma. Hypertensive and diabetic patients should be screened for respiratory function in the effort to identify cases of airway hyperreactivity or subclinical asthma. Specifically designed studies are needed to address the influence of metabolic syndrome on asthma occurrence and severity, and to unveil the potential underlying common mechanisms. Future studies will hopefully provide convincing evidence on useful therapeutic schemes that today are still unrevealed.

## REFERENCES

- 1 **Ulrik CS**, Claudius BK, Tamm M, Harving H, Siersted HC, Backer V, Helquist B, Dahl R, Høgholm A, Jøhnk IK. Effect of asthma compliance enhancement training on asthma control in patients on combination therapy with salmeterol/fluticasone propionate: a randomised controlled trial. *Clin Respir J* 2009; **3**: 161-168 [PMID: 20298399 DOI: 10.1111/j.1752-699X.2009.00129.x]
- 2 **Global Strategy for Asthma Management and Prevention**. Global Initiative for Asthma (GINA) 2012. Available from: URL: <http://www.ginasthma.org/>
- 3 **Program NAEaP**. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. *J Allergy Clin Immunol* 2007; **120**: S94-138 [PMID: 17983880 DOI: 10.1016/j.jaci.2007.09.043]
- 4 **BTS/SIGN Asthma Guideline**. British Guideline for the Management of Asthma, 2009 revision. Available from: URL: [www.brit-thoracic.org.uk](http://www.brit-thoracic.org.uk).
- 5 **Global Strategy for Asthma Management and Prevention**. Global Initiative for Asthma (GINA) 2014. Available from: URL: <http://www.ginasthma.org/>
- 6 **Demoly P**, Gueron B, Annunziata K, Adamek L, Walters RD. Update on asthma control in five European countries: results of a 2008 survey. *Eur Respir Rev* 2010; **19**: 150-157 [PMID: 20956184 DOI: 10.1183/09059180.00002110]
- 7 **Gershon AS**, Wang C, Guan J, To T. Burden of comorbidity in individuals with asthma. *Thorax* 2010; **65**: 612-618 [PMID: 20627918 DOI: 10.1136/thx.2009.131078]
- 8 **Beuther DA**, Sutherland ER. Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. *Am J Respir Crit Care Med* 2007; **175**: 661-666 [PMID: 17234901 DOI: 10.1164/rccm.200611-1717OC]
- 9 **Thuesen BH**, Husemoen LL, Hersoug LG, Pisinger C, Linneberg A. Insulin resistance as a predictor of incident asthma-like symptoms in adults. *Clin Exp Allergy* 2009; **39**: 700-707 [PMID: 19260867 DOI: 10.1111/j.1365-2222.2008.03197.x]
- 10 **Adeyeye OO**, Ogbera AO, Ogunleye OO, Brodie-Mens AT, Abolarinwa FF, Bamisile RT, Onadeko BO. Understanding asthma and the metabolic syndrome - a Nigerian report. *Int Arch Med* 2012; **5**: 20 [PMID: 22726248 DOI: 10.1186/1755-7682-5-20]
- 11 **Brumpton BM**, Camargo CA, Romundstad PR, Langhammer A, Chen Y, Mai XM. Metabolic syndrome and incidence of asthma in adults: the HUNT study. *Eur Respir J* 2013; **42**: 1495-1502 [PMID: 23845717 DOI: 10.1183/09031936.00046013]
- 12 **Park J**, Kim TB, Joo H, Lee JS, Lee SD, Oh YM. Diseases concomitant with asthma in middle-aged and elderly subjects in Korea: a population-based study. *Allergy Asthma Immunol Res* 2013; **5**: 16-25 [PMID: 23277874 DOI: 10.4168/aaair.2013.5.1.16]
- 13 **Ogden CL**, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity among adults: United States, 2011-2012. *NCHS Data Brief* 2013; **(131)**: 1-8 [PMID: 24152742]
- 14 **Cazzola M**, Segreti A, Calzetta L, Rogliani P. Comorbidities of asthma: current knowledge and future research needs. *Curr Opin Pulm Med* 2013; **19**: 36-41 [PMID: 23114561 DOI: 10.1097/MCP.0b013e32835b113a]
- 15 **Weiss ST**, Shore S. Obesity and asthma: directions for research. *Am J Respir Crit Care Med* 2004; **169**: 963-968 [PMID: 14742299 DOI: 10.1164/rccm.200303-403WS]
- 16 **Ford ES**. The epidemiology of obesity and asthma. *J Allergy Clin Immunol* 2005; **115**: 897-909; quiz 910 [PMID: 15867841 DOI: 10.1016/j.jaci.2004.11.050]
- 17 **Boulet LP**. Asthma and obesity. *Clin Exp Allergy* 2013; **43**: 8-21 [PMID: 23278876 DOI: 10.1111/j.1365-2222.2012.04040.x]
- 18 **McGinley B**, Punjabi NM. Obesity, metabolic abnormalities, and asthma: establishing causal links. *Am J Respir Crit Care Med* 2011; **183**: 424-425 [PMID: 21325079 DOI: 10.1164/rccm.201009-1525ED]
- 19 Expert Panel on Detection Ea, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; **285**: 2486-2497 [PMID: 11368702]
- 20 **Dandona P**, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. *Circulation* 2005; **111**: 1448-1454 [PMID: 15781756 DOI: 10.1161/01.CIR.0000158483.13093.9D]
- 21 **Kassi E**, Pervanidou P, Kaltsas G, Chrousos GP. Metabolic syndrome: definitions and controversies. *BMC Med* 2011; **9**: 48 [PMID: 21542944 DOI: 10.1186/1741-7015-9-48]
- 22 **Sovio U**, Skow A, Falconer C, Park MH, Viner RM, Kinra S. Improving prediction algorithms for cardiometabolic risk in children and adolescents. *J Obes* 2013; **2013**: 684782 [PMID: 23862055 DOI: 10.1155/2013/684782]
- 23 **Yarnell JW**, Patterson CC, Bainton D, Sweetnam PM. Is metabolic syndrome a discrete entity in the general population? Evidence from the Caerphilly and Speedwell population studies. *Heart* 1998; **79**: 248-252 [PMID: 9602657 DOI: 10.1136/hrt.79.3.248]
- 24 **Jensen ME**, Gibson PG, Collins CE, Wood LG. Airway and systemic inflammation in obese children with asthma. *Eur Respir J* 2013; **42**: 1012-1019 [PMID: 23349447 DOI: 10.1183/09031936.00124912]
- 25 **Garmendia JV**, Moreno D, Garcia AH, De Sanctis JB. Metabolic syndrome and asthma. *Recent Pat Endocr Metab Immune Drug Discov* 2014; **8**: 60-66 [PMID: 24397782 DOI: 10.2174/1872214807666140107151023]
- 26 **Dandona P**, Ghanim H, Monte SV, Caruana JA, Green K, Abuaysheh S, Lohano T, Schentag J, Dhindsa S, Chaudhuri A. Increase in the mediators of asthma in obesity and obesity with type 2 diabetes: reduction with weight loss. *Obesity* (Silver Spring) 2014; **22**: 356-362 [PMID: 23804543 DOI: 10.1002/oby.20524]
- 27 **Assad N**, Qualls C, Smith LJ, Arynchyn A, Thyagarajan B, Schuyler M, Jacobs DR, Sood A. Body mass index is a stronger predictor than the metabolic syndrome for future asthma in women. The longitudinal CARDIA study. *Am J Respir Crit Care Med* 2013; **188**: 319-326 [PMID: 23905525 DOI: 10.1164/rccm.201303-0457OC]
- 28 **Bel EH**. Another piece to the puzzle of the "obese female asthma" phenotype. *Am J Respir Crit Care Med* 2013; **188**: 263-264 [PMID: 23905517 DOI: 10.1164/rccm.201306-1066ED]
- 29 **Agrawal A**, Prakash YS, Linneberg A. Body mass index is not a stronger predictor than the metabolic syndrome for future asthma in women. *Am J Respir Crit Care Med* 2014; **189**: 231-232 [PMID: 24428655 DOI: 10.1164/rccm.201307-1333LE]
- 30 **Gnanell R**, Henderson AJ, Evans DM, Smith GD, Ness AR, Lewis S, Palmer TM, Sterne JA. Effects of BMI, fat mass, and lean mass

- on asthma in childhood: a Mendelian randomization study. *PLoS Med* 2014; **11**: e1001669 [PMID: 24983943 DOI: 10.1371/journal.pmed.1001669]
- 31 **Agrawal A**, Prakash YS. Obesity, metabolic syndrome, and airway disease: a bioenergetic problem? *Immunol Allergy Clin North Am* 2014; **34**: 785-796 [PMID: 25282291 DOI: 10.1016/j.iac.2014.07.004]
- 32 **Shore SA**. Obesity and asthma: lessons from animal models. *J Appl Physiol* (1985) 2007; **102**: 516-528 [PMID: 17053103]
- 33 **Shore SA**. Obesity, airway hyperresponsiveness, and inflammation. *J Appl Physiol* (1985) 2010; **108**: 735-743 [PMID: 19875711 DOI: 10.1152/jappphysiol.00847.2006]
- 34 **Del-Rio-Navarro BE**, Castro-Rodriguez JA, Garibay Nieto N, Berber A, Toussaint G, Sienra-Monge JJ, Romieu I. Higher metabolic syndrome in obese asthmatic compared to obese nonasthmatic adolescent males. *J Asthma* 2010; **47**: 501-506 [PMID: 20560825 DOI: 10.3109/02770901003702808]
- 35 **Rasmussen F**, Hancox RJ, Nair P, Hansen HS, Siersted HC, Nybo M. Associations between airway hyperresponsiveness, obesity and lipoproteins in a longitudinal cohort. *Clin Respir J* 2013; **7**: 268-275 [PMID: 22906044 DOI: 10.1111/crj.12000]
- 36 **Farah CS**, Salome CM. Asthma and obesity: a known association but unknown mechanism. *Respirology* 2012; **17**: 412-421 [PMID: 21992497 DOI: 10.1111/j.1440-1843.2011.02080.x]
- 37 **Lugogo NL**, Bappanad D, Kraft M. Obesity, metabolic dysregulation and oxidative stress in asthma. *Biochim Biophys Acta* 2011; **1810**: 1120-1126 [PMID: 21944975 DOI: 10.1016/j.bbagen.2011.09.004]
- 38 **Bruno A**, Pace E, Cibella F, Chanez P. Body mass index and comorbidities in adult severe asthmatics. *Biomed Res Int* 2014; **2014**: 607192 [PMID: 24987694 DOI: 10.1155/2014/607192]
- 39 **Sepe A**, Tchkonina T, Thomou T, Zamboni M, Kirkland JL. Aging and regional differences in fat cell progenitors - a mini-review. *Gerontology* 2011; **57**: 66-75 [PMID: 20110661 DOI: 10.1159/000279755]
- 40 **Bremer AA**, Jialal I. Adipose tissue dysfunction in nascent metabolic syndrome. *J Obes* 2013; **2013**: 393192 [PMID: 23653857 DOI: 10.1155/2013/393192]
- 41 **Staiano AE**, Katzmarzyk PT. Ethnic and sex differences in body fat and visceral and subcutaneous adiposity in children and adolescents. *Int J Obes (Lond)* 2012; **36**: 1261-1269 [PMID: 22710928 DOI: 10.1038/ijo.2012.95]
- 42 **Blüher M**. Clinical relevance of adipokines. *Diabetes Metab J* 2012; **36**: 317-327 [PMID: 23130315 DOI: 10.4093/dmj.2012.36.5.317]
- 43 **Ali Assad N**, Sood A. Leptin, adiponectin and pulmonary diseases. *Biochimie* 2012; **94**: 2180-2189 [PMID: 22445899 DOI: 10.1016/j.biochi.2012.03.006]
- 44 **Yamamoto R**, Ueki S, Moritoki Y, Kobayashi Y, Oyamada H, Konno Y, Tamaki M, Itoga M, Takeda M, Ito W, Chihara J. Adiponectin attenuates human eosinophil adhesion and chemotaxis: implications in allergic inflammation. *J Asthma* 2013; **50**: 828-835 [PMID: 23777560 DOI: 10.3109/02770903.2013.816725]
- 45 **Yamauchi T**, Iwabu M, Okada-Iwabu M, Kadowaki T. Adiponectin receptors: a review of their structure, function and how they work. *Best Pract Res Clin Endocrinol Metab* 2014; **28**: 15-23 [PMID: 24417942 DOI: 10.1016/j.beem.2013.09.003]
- 46 **Zhu XL**, Qin XQ, Xiang Y, Tan YR, Qu XP, Liu HJ. Adipokine adiponectin is a potential protector to human bronchial epithelial cell for regulating proliferation, wound repair and apoptosis: comparison with leptin and resistin. *Peptides* 2013; **40**: 34-41 [PMID: 23220445 DOI: 10.1016/j.peptides.2012.11.017]
- 47 **Ziora D**, Machura E, Ziora KT, Swietochowska E, Oswiecimska JM, Kasperska-Zajac A. Serum resistin levels are elevated in schoolchildren with atopic asthma. *Neuro Endocrinol Lett* 2013; **34**: 212-216 [PMID: 23685419]
- 48 **Rojas-Dotor S**, Segura-Méndez NH, Miyagui-Namikawa K, Mondragón-González R. Expression of resistin, CXCR3, IP-10, CCR5 and MIP-1α in obese patients with different severity of asthma. *Biol Res* 2013; **46**: 13-20 [PMID: 23760409 DOI: 10.4067/S0716-97602013000100002]
- 49 **Leivo-Korpela S**, Lehtimäki L, Vuolteenaho K, Nieminen R, Kankaanranta H, Saarelainen S, Moilanen E. Adipokine resistin predicts anti-inflammatory effect of glucocorticoids in asthma. *J Inflamm (Lond)* 2011; **8**: 12 [PMID: 21615949 DOI: 10.1186/1476-9255-8-12]
- 50 **Quek YW**, Sun HL, Ng YY, Lee HS, Yang SF, Ku MS, Lu KH, Sheu JN, Lue KH. Associations of serum leptin with atopic asthma and allergic rhinitis in children. *Am J Rhinol Allergy* 2010; **24**: 354-358 [PMID: 21244735 DOI: 10.2500/ajra.2010.24.3483]
- 51 **Sood A**, Ford ES, Camargo CA. Association between leptin and asthma in adults. *Thorax* 2006; **61**: 300-305 [PMID: 16540481 DOI: 10.1136/thx.2004.031468]
- 52 **Sarraf P**, Frederich RC, Turner EM, Ma G, Jaskowiak NT, Rivet DJ, Flier JS, Lowell BB, Fraker DL, Alexander HR. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. *J Exp Med* 1997; **185**: 171-175 [PMID: 8996253 DOI: 10.1084/jem.185.1.171]
- 53 **Guler N**, Kirceli E, Ones U, Tamay Z, Salmayenli N, Darendeliler F. Leptin: does it have any role in childhood asthma? *J Allergy Clin Immunol* 2004; **114**: 254-259 [PMID: 15316499 DOI: 10.1016/j.jaci.2004.03.053]
- 54 **Fuentes E**, Guzmán-Jofre L, Moore-Carrasco R, Palomo I. Role of PPARs in inflammatory processes associated with metabolic syndrome (Review). *Mol Med Rep* 2013; **8**: 1611-1616 [PMID: 24100795 DOI: 10.3892/mmr.2013.1714]
- 55 **Wang W**, Zhu Z, Zhu B, Ma Z. Pioglitazone attenuates allergic inflammation and induces production of regulatory T lymphocytes. *Am J Rhinol Allergy* 2010; **24**: 454-458 [PMID: 21144225 DOI: 10.2500/ajra.2010.24.3522]
- 56 **Gowdy KM**, Fessler MB. Emerging roles for cholesterol and lipoproteins in lung disease. *Pulm Pharmacol Ther* 2013; **26**: 430-437 [PMID: 22706330 DOI: 10.1016/j.pupt.2012.06.002]
- 57 **Yao X**, Remaley AT, Levine SJ. New kids on the block: the emerging role of apolipoproteins in the pathogenesis and treatment of asthma. *Chest* 2011; **140**: 1048-1054 [PMID: 21972383 DOI: 10.1378/chest.11-0158]
- 58 **Viallourous PK**, Savva SC, Kolokotroni O, Behbod B, Zeniou M, Economou M, Chadjigeorgiou C, Kourides YA, Tornaritis MJ, Lamnisos D, Middleton N, Milton DK. Low serum high-density lipoprotein cholesterol in childhood is associated with adolescent asthma. *Clin Exp Allergy* 2012; **42**: 423-432 [PMID: 22356143 DOI: 10.1111/j.1365-2222.2011.03940.x]
- 59 **Fenger RV**, Gonzalez-Quintela A, Linneberg A, Husemoen LL, Thuesen BH, Aadahl M, Vidal C, Skaaby T, Sainz JC, Calvo E. The relationship of serum triglycerides, serum HDL, and obesity to the risk of wheezing in 85,555 adults. *Respir Med* 2013; **107**: 816-824 [PMID: 23465506 DOI: 10.1016/j.rmed.2013.02.001]
- 60 **Scichilone N**, Rizzo M, Benfante A, Catania R, Giglio RV, Nikolic D, Montalto G, Bellia V. Serum low density lipoprotein subclasses in asthma. *Respir Med* 2013; **107**: 1866-1872 [PMID: 24075885 DOI: 10.1016/j.rmed.2013.09.001]
- 61 **Hoefner DM**, Hodel SD, O'Brien JF, Branum EL, Sun D, Meissner I, McConnell JP. Development of a rapid, quantitative method for LDL subfractionation with use of the Quantimetrix Lipoprint LDL System. *Clin Chem* 2001; **47**: 266-274 [PMID: 11159775]
- 62 **Singh S**, Prakash YS, Linneberg A, Agrawal A. Insulin and the lung: connecting asthma and metabolic syndrome. *J Allergy (Cairo)* 2013; **2013**: 627384 [PMID: 24204385 DOI: 10.1155/2013/627384]
- 63 **Kim JA**, Wei Y, Sowers JR. Role of mitochondrial dysfunction in insulin resistance. *Circ Res* 2008; **102**: 401-414 [PMID: 18309108 DOI: 10.1161/CIRCRESAHA.107.165472]
- 64 **Nisoli E**, Clementi E, Carruba MO, Moncada S. Defective mitochondrial biogenesis: a hallmark of the high cardiovascular risk in the metabolic syndrome? *Circ Res* 2007; **100**: 795-806 [PMID: 17395885 DOI: 10.1161/01.RES.0000259591.97107.6c]
- 65 **Bugger H**, Abel ED. Molecular mechanisms for myocardial mitochondrial dysfunction in the metabolic syndrome. *Clin Sci (Lond)* 2008; **114**: 195-210 [PMID: 18184113 DOI: 10.1042/CS20070166]
- 66 **Aroor AR**, Mandavia C, Ren J, Sowers JR, Pulakat L. Mitochondria



- and Oxidative Stress in the Cardiorenal Metabolic Syndrome. *Cardiorenal Med* 2012; **2**: 87-109 [PMID: 22619657]
- 67 **Mabaliarajan U**, Dinda AK, Kumar S, Roshan R, Gupta P, Sharma SK, Ghosh B. Mitochondrial structural changes and dysfunction are associated with experimental allergic asthma. *J Immunol* 2008; **181**: 3540-3548 [PMID: 18714027 DOI: 10.4049/jimmunol.181.5.3540]
  - 68 **Mabaliarajan U**, Rehman R, Ahmad T, Kumar S, Singh S, Leishangthem GD, Aich J, Kumar M, Khanna K, Singh VP, Dinda AK, Biswal S, Agrawal A, Ghosh B. Linoleic acid metabolite drives severe asthma by causing airway epithelial injury. *Sci Rep* 2013; **3**: 1349 [PMID: 23443229 DOI: 10.1038/srep01349]
  - 69 **Mabaliarajan U**, Dinda AK, Sharma SK, Ghosh B. Esculetin restores mitochondrial dysfunction and reduces allergic asthma features in experimental murine model. *J Immunol* 2009; **183**: 2059-2067 [PMID: 19570833 DOI: 10.4049/jimmunol.0900342]
  - 70 **Aich J**, Mabaliarajan U, Ahmad T, Khanna K, Rehman R, Agrawal A, Ghosh B. Resveratrol attenuates experimental allergic asthma in mice by restoring inositol polyphosphate 4 phosphatase (INPP4A). *Int Immunopharmacol* 2012; **14**: 438-443 [PMID: 22986054 DOI: 10.1016/j.intimp.2012.08.017]
  - 71 **Ahmad T**, Mabaliarajan U, Sharma A, Aich J, Makhija L, Ghosh B, Agrawal A. Simvastatin improves epithelial dysfunction and airway hyperresponsiveness: from asymmetric dimethyl-arginine to asthma. *Am J Respir Cell Mol Biol* 2011; **44**: 531-539 [PMID: 20558777 DOI: 10.1165/rmb.2010-0041OC]
  - 72 **Aguilera-Aguirre L**, Basci A, Saavedra-Molina A, Kurosky A, Sur S, Boldogh I. Mitochondrial dysfunction increases allergic airway inflammation. *J Immunol* 2009; **183**: 5379-5387 [PMID: 19786549 DOI: 10.4049/jimmunol.0900228]
  - 73 **Holguin F**, Fitzpatrick A. Obesity, asthma, and oxidative stress. *J Appl Physiol* (1985) 2010; **108**: 754-759 [PMID: 19926826 DOI: 10.1152/jappphysiol.00702.2009]
  - 74 **Keaney JF**, Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D, Massaro JM, Sutherland P, Vita JA, Benjamin EJ. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler Thromb Vasc Biol* 2003; **23**: 434-439 [PMID: 12615693 DOI: 10.1161/01.atv.0000058402.34138.11]
  - 75 **Sakai H**, Nishizawa Y, Nishimura A, Chiba Y, Goto K, Hanazaki M, Misawa M. Angiotensin II induces hyperresponsiveness of bronchial smooth muscle via an activation of p42/44 ERK in rats. *Pflügers Arch* 2010; **460**: 645-655 [PMID: 20495822 DOI: 10.1007/s00424-010-0844-y]
  - 76 **Ramsay SG**, Kenyon CJ, Whyte N, McKay IC, Thomson NC, Lindop GB. Effects of angiotensin II on remodelling of the airway and the vasculature in the rat. *Clin Sci (Lond)* 2000; **98**: 1-7 [PMID: 10600652]
  - 77 **Maneechotesuwan K**, Ekjitrakul W, Kasetsinsombat K, Wongkajornsilp A, Barnes PJ. Statins enhance the anti-inflammatory effects of inhaled corticosteroids in asthmatic patients through increased induction of indoleamine 2, 3-dioxygenase. *J Allergy Clin Immunol* 2010; **126**: 754-762.e1 [PMID: 20920765 DOI: 10.1016/j.jaci.2010.08.005]
  - 78 **Zeki AA**, Kenyon NJ, Goldkorn T. Statin drugs, metabolic pathways, and asthma: a therapeutic opportunity needing further research. *Drug Metab Lett* 2011; **5**: 40-44 [PMID: 21198438 DOI: 10.2174/187231211794455217]
  - 79 **Huang CC**, Chan WL, Chen YC, Chen TJ, Chou KT, Lin SJ, Chen JW, Leu HB. Statin use in patients with asthma: a nationwide population-based study. *Eur J Clin Invest* 2011; **41**: 507-512 [PMID: 21128938 DOI: 10.1111/j.1365-2362.2010.02434.x]
  - 80 **Yuan C**, Zhou L, Cheng J, Zhang J, Teng Y, Huang M, Adcock IM, Barnes PJ, Yao X. Statins as potential therapeutic drug for asthma? *Respir Res* 2012; **13**: 108 [PMID: 23176705 DOI: 10.1186/1465-9921-13-108]
  - 81 **Silva D**, Couto M, Delgado L, Moreira A. A systematic review of statin efficacy in asthma. *J Asthma* 2012; **49**: 885-894 [PMID: 23034069 DOI: 10.3109/02770903.2012.721433]
  - 82 **Moini A**, Azimi G, Farivar A. Evaluation of atorvastatin for the treatment of patients with asthma: a double-blind randomized clinical trial. *Allergy Asthma Immunol Res* 2012; **4**: 290-294 [PMID: 22950035 DOI: 10.4168/aa.2012.4.5.290]
  - 83 **Maneechotesuwan K**, Kasetsinsombat K, Wamanuttajinda V, Wongkajornsilp A, Barnes PJ. Statins enhance the effects of corticosteroids on the balance between regulatory T cells and Th17 cells. *Clin Exp Allergy* 2013; **43**: 212-222 [PMID: 23331562 DOI: 10.1111/cea.12067]
  - 84 **Menzies D**, Nair A, Meldrum KT, Fleming D, Barnes M, Lipworth BJ. Simvastatin does not exhibit therapeutic anti-inflammatory effects in asthma. *J Allergy Clin Immunol* 2007; **119**: 328-335 [PMID: 17141851 DOI: 10.1016/j.jaci.2006.10.014]
  - 85 **Si XB**, Zhang S, Huo LY, Dai WL, Wang HL. Statin therapy does not improve lung function in asthma: a meta-analysis of randomized controlled trials. *J Int Med Res* 2013; **41**: 276-283 [PMID: 23569033 DOI: 10.1177/0300060513477005]
  - 86 **Agrawal A**, Mabaliarajan U, Ahmad T, Ghosh B. Emerging interface between metabolic syndrome and asthma. *Am J Respir Cell Mol Biol* 2011; **44**: 270-275 [PMID: 20656947 DOI: 10.1165/rmb.2010-0141TR]
  - 87 **Capra V**, Rovati GE. Rosuvastatin inhibits human airway smooth muscle cells mitogenic response to eicosanoid contractile agents. *Pulm Pharmacol Ther* 2014; **27**: 10-16 [PMID: 23806820 DOI: 10.1016/j.pupt.2013.06.005]
  - 88 **Sasaki O**, Imamura M, Yamazumi Y, Harada H, Matsumoto T, Okunishi K, Nakagome K, Tanaka R, Akiyama T, Yamamoto K, Dohi M. Alendronate attenuates eosinophilic airway inflammation associated with suppression of Th2 cytokines, Th17 cytokines, and eotaxin-2. *J Immunol* 2013; **191**: 2879-2889 [PMID: 23935198 DOI: 10.4049/jimmunol.1300460]
  - 89 **Wu J**, Zhang Y, Liu Q, Zhong W, Xia Z. All-trans retinoic acid attenuates airway inflammation by inhibiting Th2 and Th17 response in experimental allergic asthma. *BMC Immunol* 2013; **14**: 28 [PMID: 23800145 DOI: 10.1186/1471-2172-14-28]
  - 90 **Tsuchiya H**, Ikeda Y, Ebata Y, Kojima C, Katsuma R, Tsuruyama T, Sakabe T, Shomori K, Komeda N, Oshiro S, Okamoto H, Takubo K, Hama S, Shudo K, Kogure K, Shiota G. Retinoids ameliorate insulin resistance in a leptin-dependent manner in mice. *Hepatology* 2012; **56**: 1319-1330 [PMID: 22531980 DOI: 10.1002/hep.25798]
  - 91 **McIlroy GD**, Delibegovic M, Owen C, Stoney PN, Shearer KD, McCaffery PJ, Mody N. Fenretinide treatment prevents diet-induced obesity in association with major alterations in retinoid homeostatic gene expression in adipose, liver, and hypothalamus. *Diabetes* 2013; **62**: 825-836 [PMID: 23193184 DOI: 10.2337/db12-0458]
  - 92 **Arron JR**, Scheerens H, Matthews JG. Redefining approaches to asthma: developing targeted biologic therapies. *Adv Pharmacol* 2013; **66**: 1-49 [PMID: 23433454 DOI: 10.1016/B978-0-12-404717-4.00001-9]
  - 93 **Garcia G**, Taillé C, Laveneziana P, Bourdin A, Chanez P, Humbert M. Anti-interleukin-5 therapy in severe asthma. *Eur Respir Rev* 2013; **22**: 251-257 [PMID: 23997052 DOI: 10.1183/09059180.0004013]
  - 94 **Ota T**. Chemokine systems link obesity to insulin resistance. *Diabetes Metab J* 2013; **37**: 165-172 [PMID: 23807918 DOI: 10.4093/dmj.2013.37.3.165]
  - 95 **Malagón MM**, Díaz-Ruiz A, Guzmán-Ruiz R, Jiménez-Gómez Y, Moreno NR, García-Navarro S, Vázquez-Martínez R, Peinado JR. Adipobiology for novel therapeutic approaches in metabolic syndrome. *Curr Vasc Pharmacol* 2013; **11**: 954-967 [PMID: 24168446 DOI: 10.2174/1570161113116660170]
  - 96 **Linderholm AL**, Bratt JM, Schuster GU, Zeki AA, Kenyon NJ. Novel therapeutic strategies for adult obese asthmatics. *Immunol Allergy Clin North Am* 2014; **34**: 809-823 [PMID: 25282293 DOI: 10.1016/j.iac.2014.07.006]
  - 97 **Rinne ST**, Feemster LC, Collins BF, Au DH, Perkins M, Bryson CL, O'Riordan TG, Liu CF. Thiazolidinediones and the risk of asthma exacerbation among patients with diabetes: a cohort study. *Allergy Asthma Clin Immunol* 2014; **10**: 34 [PMID: 25024717 DOI: 10.1186/1710-1492-10-34]



- 98 **Perez MK**, Piedimonte G. Metabolic asthma: is there a link between obesity, diabetes, and asthma? *Immunol Allergy Clin*

*North Am* 2014; **34**: 777-784 [PMID: 25282290 DOI: 10.1016/j.iac.2014.07.002]

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## Voltage gated calcium channel antibody-related neurological diseases

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is proximal muscle weakness, areflexia and autonomic dysfunction. Fifty to sixty percent of LEMS patients have a neoplasia, usually SCLC. The co-occurrence of SCLC and LEMS causes more severe and progressive disease and shorter survival than non-paraneoplastic LEMS. Treatment includes 3,4 diaminopyridine for symptomatic purposes and immunotherapy with prednisolone, azathioprine or intravenous immunoglobulin in patients unresponsive to 3,4 diaminopyridine. Paraneoplastic cerebellar degeneration (PCD) is a syndrome characterized with severe, subacute pancerebellar dysfunction. Serum is positive for VGCC antibody in 41%-44% of patients, usually with the co-occurrence of SCLC. Clinical and electrophysiological features of LEMS are also present in 20%-40% of these patients. Unfortunately, PCD symptoms do not improve with immunotherapy. The role of VGCC antibody in the immunopathogenesis of LEMS is well known whereas its role in PCD is still unclear. All patients presenting with LEMS or PCD must be investigated for SCLC.

**Key words:** Voltage gated calcium channel antibody; Lambert-Eaton myasthenic syndrome; Paraneoplastic cerebellar degeneration; Onconeural antibodies; Small cell lung cancer

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### Abstract

Voltage gated calcium channel (VGCC) antibodies are generally associated with Lambert-Eaton myasthenic syndrome. However the presence of this antibody has been associated with paraneoplastic as well as non-paraneoplastic cerebellar degeneration. Most patients with VGCC-antibody-positivity have small cell lung cancer (SCLC). Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune disease of the presynaptic part of the neuromuscular junction. Its classical clinical triad

**Core tip:** Voltage gated calcium channel (VGCC) antibodies are generally associated with Lambert-Eaton myasthenic syndrome, but also with paraneoplastic or non-paraneoplastic cerebellar degeneration. The autoimmune nature of non-tumour Lambert-Eaton myasthenic syndrome is reflected in its association with various HLA subtypes and other autoimmune diseases such as vitiligo, myasthenia gravis and diabetes mellitus. The most common tumour associated with VGCC-antibody-positivity is small cell lung cancer. Knowledge on the relation between cerebellar degeneration and VGCC is limited, and treatment

response is poor in this group of patients.

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## INTRODUCTION

Voltage gated calcium channels are immunologic targets for several disease. The calcium channels as a target of the pathogenic antibodies in Lambert-Eaton myasthenic syndrome (LEMS) was first suggested by Fukunaga *et al*<sup>[1]</sup> in 1983. Subsequent studies showed antibodies against P/Q type calcium channel as the most prominent in these patients<sup>[2]</sup>.

Although voltage gated calcium channel (VGCC) antibodies are generally associated with LEMS, usually seen as a paraneoplastic syndrome with small cell lung cancer (SCLC), rarely non-paraneoplastic cerebellar degeneration may also occur in the presence of this antibody<sup>[3,4]</sup>. VGCC antibody positivity is observed in 85%-90% of LEMS patients whereas the ratio approaches 100% in LEMS patients with SCLC<sup>[5]</sup>. Approximately 40% of patients with subacute onset cerebellar degeneration, usually with SCLC, have VGCC antibody positivity<sup>[3,6]</sup>. Moreover these antibodies can also be detected in SCLC patients without neurological involvement<sup>[5]</sup>.

## VGCC

The VGCC is crucial in the depolarization of the cell membrane and cellular influx of calcium in response to action potential. It functions as a secondary messenger in electrical signalization and initiates several cellular mechanisms<sup>[7]</sup>. They are found in several cells, such as smooth and skeletal muscle fibers, endocrine cells, neurons<sup>[7]</sup>. The channel also locates on the presynaptic membrane of the axon terminal. VGCC opens by action potential and leads to the entry of calcium ions into the axon terminals. Calcium influx results in movement of acetylcholine vesicles towards the presynaptic membrane and acetylcholine is released into the synaptic cleft. In striated muscles, the VGCC on the membrane of transverse tubules directly activates ryanodine-sensitive calcium channels in the sarcoplasmic reticulum and initiates rapid contraction<sup>[7,8]</sup>.

VGCC is divided into five types: L, P/Q, N, R, T depending on tissues and pharmacological properties<sup>[7]</sup>. The channel contains 4 or 5 subunits ( $\alpha 1$ ,  $\alpha 2/\delta$ ,  $\beta$  and  $\gamma$ ). The ion transition pore responsible for the biochemical and electrophysiological properties is the  $\alpha 1$  subunit. This subunit contains six helical

transmembrane segments (S1-S6) and 4 domains (I-IV)<sup>[9]</sup> (Figure 1). Ten different  $\alpha 1$  subunits have been defined and Cav2.1  $\alpha 1$  subunit is found in P/Q type VGCC<sup>[7]</sup>. Voltage sensors are located in the S4 segment. The S5 and S6 segments are sensitive to calcium<sup>[9]</sup>. Antibodies against the S5-6 segments of  $\alpha 1$  subunit are detected in 50% of LEMS patients<sup>[5]</sup>. Other antibodies detected in LEMS patients are against domain IV and  $\beta$  subunit<sup>[5,10]</sup>. However, the pathogenic role of  $\beta$  subunit antibodies is still controversial due to its intracellular location.

Antibodies to P/Q type channels are responsible for clinical symptoms of LEMS<sup>[5]</sup>. Thirty to forty percent of the patients with antibodies to P/Q type channels also have antibodies to N-type channels whereas in 25% patients also have antibodies to L-type channels<sup>[5]</sup>. Antibodies to N and P/Q type channels are detected in 40% of patients with cerebellar ataxia associated with SCLC<sup>[9]</sup>. Sry-like high-mobility group box (SOX-1), zic-4, anti-Hu are other antibodies detected in the sera of patients with PCD and SCLC and approximately 70% of the patients have one of these antibodies<sup>[6]</sup>.

## LEMS

LEMS is the autoimmune disease of the presynaptic nerve terminals. It is a rare disease with a prevalence of 2.3 per million and an incidence of 0.5 per million<sup>[11]</sup>. It is associated with SCLC in 50%-60% of patients. As the clinical and laboratory features differ in patients with and without SCLC, the disease is divided into two groups as LEMS with SCLC (SCLC-LEMS) and non-tumour (NT-LEMS). The age of onset is 50 years or above and there is a male predominance in patients with SCLC-LEMS. On the other hand NT-LEMS can be seen in all age groups with a peak at the age of 35 and 60 and a female predominance<sup>[12]</sup>.

LEMS hardly occurs in childhood; only 5% of LEMS patients are less than 18-year-old<sup>[13]</sup>. Our youngest LEMS patient was a eight year-old female.

## Pathogenesis

LEMS is a disorder due to antibodies against P/Q type VGCC. VGCC antibodies interact with extracellular S5 and S6 segments of domain II, III and IV of  $\alpha 1$  subunit and reduce the number of ion channels by cross binding<sup>[5,14-16]</sup>. The antibodies can also bind to other VGCC types without causing any dysfunction. Although VGCC antibodies usually generate an immune reaction, the response to epitopes varies in LEMS patients<sup>[17]</sup>. Antigenic modulation followed by clustering and reduction of VGCC leads to reduction in quantal release of acetylcholine in synapses and results in muscle weakness<sup>[18]</sup>. The down-regulation of the receptors of parasympathetic and sympathetic neurons that cause autonomic dysfunction is also associated with these antibodies.

VGCC antibodies can be detected by radioimmunoassay

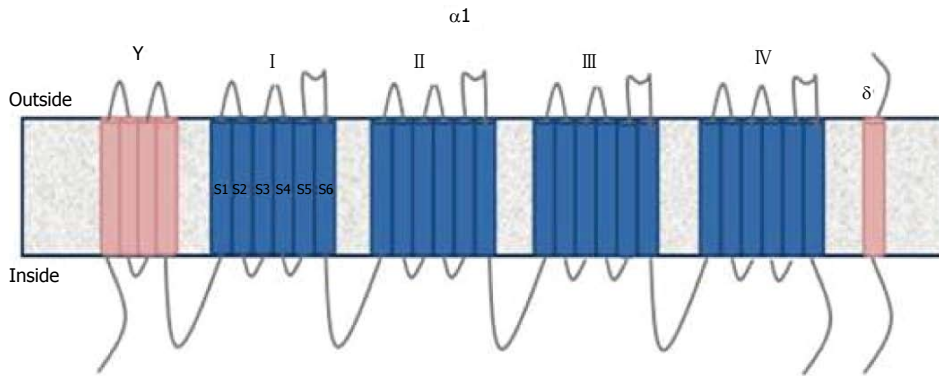


Figure 1 The structure of Voltage gated calcium channels.

in 85%-90% of LEMS patients and 100% of patients with SCLC-LEMS<sup>[17,19,20]</sup>. P/Q type VGCC are expressed on SCLC cells and this expression results in production of antibodies and cross-reaction with presynaptic VGCC<sup>[21]</sup>. As the immune reaction is initiated early in tumour development, the diagnosis of LEMS frequently precedes the diagnosis of the SCLC<sup>[16]</sup>. Besides, VGCC antibodies may be detected in 3%-5% of patients with SCLC without muscle weakness or autonomic dysfunction<sup>[16]</sup>.

Approximately 10%-15% of LEMS patients do not have serum antibodies. However, their serum reproduces LEMS-like symptoms when it transferred to mice. This finding suggests the presence of antibodies at very low concentrations or antibodies against different epitopes of the VGCC that are undetected by routine tests<sup>[5]</sup>. Antibodies against synaptotagmin, a synaptic vesicle protein, have been found in some seronegative and seropositive LEMS patients<sup>[14,22]</sup>. Although there is no evidence on their pathogenic role, the blocking of synaptotagmin, which is a  $\text{Ca}^{2+}$  sensor in the membrane of the pre-synaptic axon terminal may explain the muscle weakness in LEMS<sup>[23]</sup>. Another such antibody, whose pathogenic role is unclear, is against "sry-like high-mobility group box" (SOX-1) proteins. This antibody is positive in 67% of SCLC-LEMS patients, 22%-32% of patients with SCLC without LEMS and only in 5% of NT-LEMS patients<sup>[19]</sup>.

The role of T lymphocytes in LEMS pathogenesis is unclear. Thymus and other lymphoid organs involved in myasthenia gravis (MG) do not present abnormalities in LEMS and the presynaptic terminals do not reveal T lymphocyte collection<sup>[16]</sup>. However regulatory T lymphocytes are down-regulated in SCLC-LEMS patients, but not in SCLC patients without LEMS, which suggests the dysfunction of regulatory T lymphocyte in SCLC-LEMS pathogenesis<sup>[24]</sup>.

Unlike SCLC-LEMS patients, the trigger of the immune response is not defined in NT-LEMS. Various HLA associations, also reported with other autoimmune diseases, have been documented: Approximately two-third of patients have HLA-B8 (HLA class I) and HLA-DR3, -DQ2 (HLA class II) alleles<sup>[25,26]</sup>. Supporting this phenomenon, monozygous twins have been reported

where one has LEMS and VGCC antibodies, and the other, myasthenia gravis and acetylcholine receptor antibodies<sup>[27]</sup>. On the other hand, HLA association was not found in SCLC-LEMS patients: because tumoral cells do not have strong HLA class I antigen expression, these molecules are possibly not involved in the immunopathogenesis<sup>[25]</sup>. These observations resemble the difference between MG with and without thymoma and reveal the existence of different autoimmune mechanisms in paraneoplastic disorders<sup>[16]</sup>.

### Clinical features

The classical triad of LEMS is proximal weakness, reduced tendon reflexes and autonomic dysfunction<sup>[16]</sup>.

Proximal weakness, more prominent in lower extremities, is the first symptom in 80% of patients<sup>[16,28]</sup>. In the course of the disease, 80% of patients suffer from proximal weakness in both upper and lower extremities<sup>[28,29]</sup>. Facial, bulbar and distal weakness are also frequent<sup>[16]</sup>. The weakness is more severe and rapidly progressive in SCLC-LEMS patients<sup>[16,28]</sup>.

Autonomic dysfunction, observed in 80%-96% of patients, is the most common symptom although it is less disturbing than muscle weakness<sup>[12,28]</sup>. Erectile dysfunction and constipation are more frequent symptoms than urinary retention, dry eye and reduced sweating<sup>[5]</sup>. The rate and the nature of autonomic symptoms do not differ between SCLC-LEMS and NT-LEMS<sup>[16]</sup>.

On neurological examination deep tendon reflexes are generally reduced or absent. However maximal isometric contraction of the muscle for 10 to 15 s may lead to the appearance of previously depressed or absent deep tendon reflexes and temporarily improve muscle strength, which is called "Post-exercise facilitation". This phenomenon, a characteristic feature of LEMS, is not sensitive<sup>[5]</sup>. This phenomenon may also mask the reduction of deep tendon reflexes in 40% of patients so the neurological examination must be repeated after a resting period to verify the diagnosis<sup>[5]</sup>.

### Associated diseases

The most common co-existence of LEMS is with



SCLC, reported in 50%-60% of LEMS patients<sup>[12]</sup>. Besides, 0.5%-3% of SCLC patients have LEMS<sup>[25]</sup>. This co-existence results in a more severe and rapidly progressive neurological disease with shortened survival compared to NT-LEMS which has a near normal survival<sup>[16]</sup>. Moreover SCLC-LEMS patients tend to be younger than other SCLC patients<sup>[19]</sup>.

Older age, tobacco use, increased ESR support the probability of underlying SCLC. In a Dutch-English cohort study a scoring system called DELTA-P was implemented to predict SCLC in LEMS<sup>[12]</sup>. According to DELTA-P, dysarthria, dysphagia, chewing or neck weakness(D), erectile dysfunction (E), loss of weight (L), tobacco use (T), age of onset more than 50 (A) and Karnofsky performance less than 60 (P) have a predictive value: patients with a DELTA-P score three or more have higher than 94% risk of having SCLC.

The distinction of SCLC-LEMS from NT-LEMS is important, as treatment options and outcome are different. For this reason, new markers to diagnose SCLC-LEMS are still under investigation. Although SOX-1 antibodies are detected in half of the SCLC-LEMS patients, the absence of a commercial kit, and the presence of these antibodies in NT-LEMS limit their use for differential diagnosis<sup>[30]</sup>. Another study revealed that VGCC antibody against domain IV positivity is more common in NT-LEMS patients than in SCLC-LEMS patients, but this is not used for clinical purposes yet<sup>[17]</sup>.

The co-existence of LEMS with other malignancies such as non-small cell lung cancer, prostate carcinoma, orthymoma has been reported rarely<sup>[13]</sup> and a random association could not be excluded<sup>[5]</sup>.

In children, the disease is generally non-paraneoplastic; lymphoproliferative malignancy and neuroblastoma are rare associations<sup>[13,31]</sup>. In accordance with the literature, our pediatric patient did not have any neoplasm.

Other autoimmune diseases such as thyroid disorders, alopecia, diabetes mellitus, MG can occur in NT-LEMS patients probably due to the presence of various HLA subtypes contributing to autoimmunity<sup>[32]</sup>. In our clinical experience, NT-LEMS patients may have associated vitiligo and myasthenia.

## Diagnosis

The time lag between the onset of the symptoms and diagnosis is approximately 4 mo in SCLC-LEMS and 12 mo in NT-LEMS<sup>[12,29]</sup>.

In patients with suspected LEMS, electrophysiological studies with repetitive nerve stimulation are among the most important diagnostic tests. The compound muscle action potential (CMAP) is low after the first stimulation and decreases further after repetitive stimulation at 2-5 Hz<sup>[33]</sup>. At least 10% reduction in CMAP after low frequency stimulation is considered abnormal and observed in 94%-98% of patients<sup>[34,35]</sup>. However, this finding may also be present in MG patients. To distinguish these two diseases, nerve stimulation at

high frequency (50 Hz) or, as a less painful method for the patient, post-exercise measurement is employed, which increases CMAP by more than 100% in LEMS patients<sup>[34,35]</sup>. Optimum results will be obtained if treatment is interrupted 12 h before the study and the muscle temperature is above 32 °C.

Although single fiber EMG is a sensitive test, it is used in combination with other tests as it cannot distinguish between MG and LEMS<sup>[33]</sup>. LEMS patients have increased jitter like MG patients. In case of severe neuromuscular junction dysfunction, the conduction defect in muscle fiber causes a decrease in amplitude and duration of motor unit potential as in myopathies<sup>[36]</sup>.

VGCC antibodies are detected by RIA in 85%-90% of LEMS and in almost 100% of SCLC-LEMS patients<sup>[17,19,20]</sup>. Although the presence of VGCC antibodies supports the diagnosis of LEMS, the absence of antibodies in a patient with typical clinical features does not exclude the diagnosis.

In 50% of patients SCLC-LEMS, LEMS symptoms precede the diagnosis of SCLC. In addition to scoring systems such as DELTA-P, all patients with the diagnosis of LEMS must undergo computerized tomography of the thorax and positron emission tomography (PET). If the results are negative, the screening must be repeated every 3-6 mo until the second year of the disease<sup>[37]</sup>.

The differential diagnosis of LEMS from seronegative and atypical myasthenia gravis can be challenging. Some clinical findings may be helpful: the progression of weakness is in the craniocaudal direction in MG and the reverse in LEMS; ptosis and facial weakness are less common and severe in LEMS. Electrophysiological studies described above and serological findings assist the clinician in the differential diagnosis.

LEMS with a subacute course can be misdiagnosed as Guillain Barré syndrome (GBS); the presence of sensorial symptoms, neuropathic pain, and elevated CSF protein favor the diagnosis of GBS. Amyotrophic lateral sclerosis may constitute another differential diagnosis, and can be distinguished by the asymmetrical weakness starting in the upper extremities and the presence of upper motor neuron signs.

## Treatment

The first choice for symptomatic treatment is 3,4 diaminopyridine<sup>[38]</sup>. This molecule blocks presynaptic voltage-gated potassium channels and provides a prolonged action potential which increases the quantal release of synaptic acetylcholine<sup>[39]</sup>. All randomized controlled studies of 3,4 diaminopyridine showed improvement in muscle strength and CMAP amplitudes. The drug is well tolerated although adverse effects like perioral and digital paresthesias and gastrointestinal symptoms are not uncommon. Seizures, which is the most frequent severe side effect, have been reported at high doses exceeding 100 mg/d<sup>[40,41]</sup>. In our experience, the drug is well tolerated and improves muscle

strength at the dose of 40-60 mg/d.

Other treatments, which can increase the concentration of acetylcholine in synaptic cleft, are pyridostigmine and acetylcholine esterase inhibitors but they are not as effective as they are in MG patients<sup>[42]</sup>.

In case of limited response to 3,4 diaminopyridine, immunosuppressive treatments must be considered. The combination of prednisolone and azathioprine is well studied and documented in LEMS patients<sup>[38,42]</sup>. Although there is not sufficient data, mycophenolate mofetil, cyclosporine and rituximab are also drugs employed in LEMS treatment<sup>[16,43]</sup>. Intravenous immunoglobulin (IVIg), another treatment option in paraneoplastic syndromes and MG, can also be used in LEMS. European Federation of Neurological Societies (EFNS) guidelines recommend IVIg in both SCLC-LEMS and NT-LEMS<sup>[44]</sup>. IVIg is also recommended in pregnant patients, as transplacental transmission of IgG antibodies may cause neonatal LEMS<sup>[45]</sup>. IVIg is generally preferred as its side effects are rare and it is easily used for the maintenance treatment, which is usually needed in LEMS patients. Plasma exchange whose effect is comparable to IVIg may carry technical difficulties and slightly higher rate of complications<sup>[46]</sup>.

In patients with SCLC, the treatment of the tumour is crucial. The survival of SCLC-LEMS patients is better than in patients with SCLC alone, but there is no relation with VGCC or SOX-1 antibody positivity and survival<sup>[17,19]</sup>. The better prognosis in these patients may be correlated with the diagnosis time that is earlier in LEMS patients<sup>[12,29]</sup>. Moreover HLA-B8 positivity is related to prolonged survival in SCLC-LEMS patients<sup>[47]</sup>.

Maintenance of optimal body weight, rehabilitation, frequent examinations for complications such as respiratory infections, and avoidance of drugs impairing neuromuscular transmission are other important aspects of the treatment.

## CEREBELLAR DEGENERATION ASSOCIATED WITH VGCC ANTIBODY

Paraneoplastic cerebellar degeneration (PCD) is a syndrome characterized by subacute cerebellar dysfunction<sup>[48]</sup>. Clinical and pathological features of the syndrome were described by Brain and Wilkinson<sup>[49]</sup> in 1965 by the evaluation of 13 patients and 6 autopsy cases. Diffuse loss of Purkinje cells is the pathologic hallmark of the disease and usually accompanied by thinning of granular and molecular layers, degeneration of long tracts of spinal cord, dentate and olivary nuclei<sup>[4]</sup>.

Most common neoplasms associated with cerebellar degeneration are lung, breast, ovarian cancers and Hodgkin lymphoma<sup>[48]</sup>. Onconeural antibodies such as anti-Hu, anti-Yo, anti-Ri, anti-CV2, anti-Tr, anti-Ma, anti-Ta, anti-zic 4, and anti-mGluR1 as well as VGCC antibody can be detected in PCD<sup>[50]</sup>. Clinical presentation, neuropathological findings and treatment

responses of patients vary according to the type of the onconeural antibody, suggesting distinct immune mechanisms related to different antibodies<sup>[4]</sup>.

## PATHOGENESIS

Antibodies against VGCC of the P/Q type or N type are found in 41%-44% of PCD patients, generally associated with SCLC<sup>[3,6,9]</sup>. The P/Q type VGCC is highly expressed in cerebellar Purkinje cells and in the molecular layer of the cerebellum<sup>[9,51]</sup>. About 20%-40% of these patients also have clinical or electrophysiological diagnosis of LEMS<sup>[3,6]</sup>. Neuropathological findings of PCD with LEMS (PCD-LEMS) were reported in 1973 by Satoyoshi *et al.*<sup>[51]</sup> for the first time. In a postmortem study of three PCD-LEMS patients with VGCC antibodies, 70%-80% of reduction in P/Q type VGCC of the molecular layer; loss of Purkinje cells and gliosis in the cerebellar cortex were observed<sup>[9,51]</sup>. The role of VGCC antibodies in the pathogenesis of PCD is still unclear. In a recent experimental study, antibodies of the IgG type purified from the serum of two VGCC antibody-positive patients with SCLC, one with PCD-LEMS and another patient with isolated LEMS were given to mice intrathecally, the antibodies associated with PCD-LEMS but not from isolated LEMS patients caused cerebellar ataxia in mice<sup>[52]</sup>. This finding suggests the presence of different epitopes of P/Q type VGCC antibodies which inhibit VGCC's function in cerebellum, or of other additional, yet undiscovered pathogenic antibodies<sup>[52]</sup>.

## CLINICAL, LABORATORY AND RADIOLOGICAL FEATURES

Subacute and rapidly progressive gait unsteadiness is the presenting symptom of cerebellar degeneration<sup>[50]</sup>. Gait and limb ataxia, diplopia, dysarthria are the other prominent symptoms<sup>[50]</sup>. Sometimes these complaints may precede by dizziness, nausea and viral infection-like symptoms<sup>[50]</sup>. Occasionally other signs and symptoms such as dysphagia, nystagmus and sensory deficits can be also seen during the course. Patients who had concomitant LEMS may also show proximal weakness and autonomic symptoms in addition to cerebellar symptoms<sup>[4]</sup>.

The cerebrospinal fluid (CSF) may show mild lymphocytic pleocytosis with elevated protein and oligoclonal bands<sup>[48]</sup>. VGCC antibodies may also be detected in CSF and there is some evidence of intrathecal synthesis of the antibodies, and detected in about 25% of the patients<sup>[3]</sup>. This low percentage may be explained by the absence of CSF analysis in some cases and further studies are needed to increase the rate of antibody presence in CSF.

Initial brain magnetic resonance images or tomography are normal in most patients<sup>[4]</sup> although in early stages of the disease fluorodeoxyglucose-PET scans may show cerebellar hypermetabolism<sup>[50,53]</sup>, whereas cerebellar

atrophy and cerebellar hypometabolism are seen in the advanced stage of the disease<sup>[50]</sup>.

## TREATMENT

Treatment of the underlying malignancy has the priority like other paraneoplastic syndromes<sup>[50]</sup>. Corticosteroids, plasma exchange, IVIG, tacrolimus and cyclophosphamide are the immunotherapeutic options to be used concurrently with tumour therapy, but most of the cases did not show sufficient improvement despite treatment<sup>[50]</sup>. Unlike in LEMS, immunotherapy does not result in symptomatic improvement in PCD: this suggests PCD may be associated with irreversible damage of Purkinje cells<sup>[3]</sup>.

## NON-PARANEOPLASTIC CEREBELLAR DEGENERATION WITH VGCC ANTIBODY

VGCC antibodies were also found in a few patients with non-paraneoplastic cerebellar degeneration. In a study of the antibody profile of 67 cases with sporadic, late-onset cerebellar ataxia of unknown etiology, VGCC antibodies were found in 12%<sup>[54]</sup>. Two cases with NT-LEMS who developed cerebellar ataxia during the course of their disease had VGCC antibodies in serum and CSF. Cerebellar symptoms of these patients showed no improvement with different immunotherapies, as in PCD<sup>[55]</sup>. However a few cases of non-paraneoplastic cerebellar degeneration showed favorable outcome under rituximab and IVIg treatment<sup>[56,57]</sup>.

## CONCLUSION

Diseases related to VGCC antibodies are usually associated with SCLC. Therefore, SCLC should be investigated in patients with LEMS and/or cerebellar degeneration. The role of VGCC antibodies in the immunopathogenesis of LEMS is clear, however their role in cerebellar degeneration is not known. Determination of the effect of VGCC antibodies on the pathogenesis of cerebellar degeneration may contribute to the design of more efficient treatment strategies. Therefore, experimental models and pathologic studies that investigate the effect of immune mechanisms at molecular level in the tissue are needed.

## REFERENCES

- 1 **Fukunaga H**, Engel AG, Lang B, Newsom-Davis J, Vincent A. Passive transfer of Lambert-Eaton myasthenic syndrome with IgG from man to mouse depletes the presynaptic membrane active zones. *Proc Natl Acad Sci USA* 1983; **80**: 7636-7640 [PMID: 6584877]
- 2 **Lennon VA**, Kryzer TJ, Griesmann GE, O'Suilleabhain PE, Windebank AJ, Woppmann A, Miljanich GP, Lambert EH. Calcium-channel antibodies in the Lambert-Eaton syndrome and other paraneoplastic syndromes. *N Engl J Med* 1995; **332**: 1467-1474 [PMID: 7739683]
- 3 **Graus F**, Lang B, Pozo-Rosich P, Saiz A, Casamitjana R, Vincent A. P/Q type calcium-channel antibodies in paraneoplastic cerebellar degeneration with lung cancer. *Neurology* 2002; **59**: 764-766 [PMID: 12221175]
- 4 **Mason WP**, Graus F, Lang B, Honnorat J, Delattre JY, Valdeoriola F, Antoine JC, Rosenblum MK, Rosenfeld MR, Newsom-Davis J, Posner JB, Dalmau J. Small-cell lung cancer, paraneoplastic cerebellar degeneration and the Lambert-Eaton myasthenic syndrome. *Brain* 1997; **120** (Pt 8): 1279-1300 [PMID: 9278623]
- 5 **Titulaer MJ**, Lang B, Verschuuren JJ. Lambert-Eaton myasthenic syndrome: from clinical characteristics to therapeutic strategies. *Lancet Neurol* 2011; **10**: 1098-1107 [PMID: 22094130]
- 6 **Sabater L**, Höftberger R, Boronat A, Saiz A, Dalmau J, Graus F. Antibody repertoire in paraneoplastic cerebellar degeneration and small cell lung cancer. *PLoS One* 2013; **8**: e60438 [PMID: 23536908]
- 7 **Catterall WA**. Voltage-gated calcium channels. *Cold Spring Harb Perspect Biol* 2011; **3**: a003947 [PMID: 21746798]
- 8 **Tsien RW**. Calcium channels in excitable cell membranes. *Annu Rev Physiol* 1983; **45**: 341-358 [PMID: 6303205]
- 9 **van Coevorden-Hameete MH**, de Graaff E, Titulaer MJ, Hoogenraad CC, Sillevs Smitt PA. Molecular and cellular mechanisms underlying anti-neuronal antibody mediated disorders of the central nervous system. *Autoimmun Rev* 2014; **13**: 299-312 [PMID: 24225076]
- 10 **Verschuuren JJ**, Dalmau J, Tunkel R, Lang B, Graus F, Schramm L, Posner JB, Newsom-Davis J, Rosenfeld MR. Antibodies against the calcium channel beta-subunit in Lambert-Eaton myasthenic syndrome. *Neurology* 1998; **50**: 475-479 [PMID: 9484375]
- 11 **Wirtz PW**, Nijhuis MG, Sotodeh M, Willems LN, Brahim JJ, Putter H, Wintzen AR, Verschuuren JJ. The epidemiology of myasthenia gravis, Lambert-Eaton myasthenic syndrome and their associated tumours in the northern part of the province of South Holland. *J Neurol* 2003; **250**: 698-701 [PMID: 12796832]
- 12 **Titulaer MJ**, Maddison P, Sont JK, Wirtz PW, Hilton-Jones D, Klooster R, Willcox N, Potman M, Sillevs Smitt PA, Kuks JB, Roep BO, Vincent A, van der Maarel SM, van Dijk JG, Lang B, Verschuuren JJ. Clinical Dutch-English Lambert-Eaton Myasthenic syndrome (LEMS) tumor association prediction score accurately predicts small-cell lung cancer in the LEMS. *J Clin Oncol* 2011; **29**: 902-908 [PMID: 21245427]
- 13 **Wirtz PW**, Smallegange TM, Wintzen AR, Verschuuren JJ. Differences in clinical features between the Lambert-Eaton myasthenic syndrome with and without cancer: an analysis of 227 published cases. *Clin Neurol Neurosurg* 2002; **104**: 359-363 [PMID: 12140105]
- 14 **Takamori M**, Takahashi M, Yasukawa Y, Iwasa K, Nemoto Y, Suenaga A, Nagataki S, Nakamura T. Antibodies to recombinant synaptotagmin and calcium channel subtypes in Lambert-Eaton myasthenic syndrome. *J Neurol Sci* 1995; **133**: 95-101 [PMID: 8583238]
- 15 **Iwasa K**, Takamori M, Komai K, Mori Y. Recombinant calcium channel is recognized by Lambert-Eaton myasthenic syndrome antibodies. *Neurology* 2000; **54**: 757-759 [PMID: 10680821]
- 16 **Gilhus NE**. Lambert-eaton myasthenic syndrome; pathogenesis, diagnosis, and therapy. *Autoimmune Dis* 2011; **2011**: 973808 [PMID: 21969911]
- 17 **Pellkofer HL**, Armbruster L, Krumbholz M, Titulaer MJ, Verschuuren JJ, Schumm F, Voltz R. Lambert-eaton myasthenic syndrome differential reactivity of tumor versus non-tumor patients to subunits of the voltage-gated calcium channel. *J Neuroimmunol* 2008; **204**: 136-139 [PMID: 18809213]
- 18 **Quartel A**, Turbeville S, Lounsbury D. Current therapy for Lambert-Eaton myasthenic syndrome: development of 3,4-diaminopyridine phosphate salt as first-line symptomatic treatment. *Curr Med Res Opin* 2010; **26**: 1363-1375 [PMID: 20377318]
- 19 **Titulaer MJ**, Klooster R, Potman M, Sabater L, Graus F, Hegeman IM, Thijssen PE, Wirtz PW, Twijnstra A, Smitt PA, van der Maarel



- SM, Verschuuren JJ. SOX antibodies in small-cell lung cancer and Lambert-Eaton myasthenic syndrome: frequency and relation with survival. *J Clin Oncol* 2009; **27**: 4260-4267 [PMID: 19667272]
- 20 **Motomura M**, Lang B, Johnston I, Palace J, Vincent A, Newsom-Davis J. Incidence of serum anti-P/Q-type and anti-N-type calcium channel autoantibodies in the Lambert-Eaton myasthenic syndrome. *J Neurol Sci* 1997; **147**: 35-42 [PMID: 9094058]
  - 21 **Roberts A**, Perera S, Lang B, Vincent A, Newsom-Davis J. Paraneoplastic myasthenic syndrome IgG inhibits 45Ca<sup>2+</sup> flux in a human small cell carcinoma line. *Nature* 1985; **317**: 737-739 [PMID: 2414666]
  - 22 **Takamori M**, Hamada T, Komai K, Takahashi M, Yoshida A. Synaptotagmin can cause an immune-mediated model of Lambert-Eaton myasthenic syndrome in rats. *Ann Neurol* 1994; **35**: 74-80 [PMID: 8285596]
  - 23 **Fukuda M**, Moreira JE, Liu V, Sugimori M, Mikoshiba K, Llinás RR. Role of the conserved WHXL motif in the C terminus of synaptotagmin in synaptic vesicle docking. *Proc Natl Acad Sci USA* 2000; **97**: 14715-14719 [PMID: 11114192]
  - 24 **Tani T**, Tanaka K, Idezuka J, Nishizawa M. Regulatory T cells in paraneoplastic neurological syndromes. *J Neuroimmunol* 2008; **196**: 166-169 [PMID: 18455243]
  - 25 **Titulaer MJ**, Verschuuren JJ. Lambert-Eaton myasthenic syndrome: tumor versus nontumor forms. *Ann N Y Acad Sci* 2008; **1132**: 129-134 [PMID: 18567862]
  - 26 **Wirtz PW**, Roep BO, Schreuder GM, van Doorn PA, van Engelen BG, Kuks JB, Twijnstra A, de Visser M, Visser LH, Wokke JH, Wintzen AR, Verschuuren JJ. HLA class I and II in Lambert-Eaton myasthenic syndrome without associated tumor. *Hum Immunol* 2001; **62**: 809-813 [PMID: 11476904]
  - 27 **Punga AR**, Nygren I, Askmark H, Stålberg EV. Monozygous twins with neuromuscular transmission defects at opposite sides of the motor endplate. *Acta Neurol Scand* 2009; **119**: 207-211 [PMID: 18684214]
  - 28 **Titulaer MJ**, Wirtz PW, Kuks JB, Schelhaas HJ, van der Kooi AJ, Faber CG, van der Pol WL, de Visser M, Sillevius Smitt PA, Verschuuren JJ. The Lambert-Eaton myasthenic syndrome 1988-2008: a clinical picture in 97 patients. *J Neuroimmunol* 2008; **201-202**: 153-158 [PMID: 18644631]
  - 29 **Pellkofer HL**, Armbruster L, Linke R, Schumm F, Voltz R. Managing non-paraneoplastic Lambert-Eaton myasthenic syndrome: clinical characteristics in 25 German patients. *J Neuroimmunol* 2009; **217**: 90-94 [PMID: 19833394]
  - 30 **Sabater L**, Titulaer M, Saiz A, Verschuuren J, Güre AO, Graus F. SOX1 antibodies are markers of paraneoplastic Lambert-Eaton myasthenic syndrome. *Neurology* 2008; **70**: 924-928 [PMID: 18032743]
  - 31 **Bosdure E**, Attarian S, Mancini J, Mikaeloff Y, Chabrol B. [Lambert-Eaton myasthenic syndrome revealing neuroblastoma in 2 children]. *Arch Pediatr* 2006; **13**: 1121-1124 [PMID: 16793244]
  - 32 **Wirtz PW**, Bradshaw J, Wintzen AR, Verschuuren JJ. Associated autoimmune diseases in patients with the Lambert-Eaton myasthenic syndrome and their families. *J Neurol* 2004; **251**: 1255-1259 [PMID: 15503107]
  - 33 **Medicine AQACAAoE**. Practice parameter for repetitive nerve stimulation and single fiber EMG evaluation of adults with suspected myasthenia gravis or Lambert-Eaton myasthenic syndrome: summary statement. *Muscle Nerve* 2001; **24**: 1236-1238 [PMID: 11494280]
  - 34 **Oh SJ**, Kurokawa K, Claussen GC, Ryan HF. Electrophysiological diagnostic criteria of Lambert-Eaton myasthenic syndrome. *Muscle Nerve* 2005; **32**: 515-520 [PMID: 16003742]
  - 35 **Tim RW**, Massey JM, Sanders DB. Lambert-Eaton myasthenic syndrome (LEMS). Clinical and electrodiagnostic features and response to therapy in 59 patients. *Ann N Y Acad Sci* 1998; **841**: 823-826 [PMID: 9668336]
  - 36 **O'Neill JH**, Murray NM, Newsom-Davis J. The Lambert-Eaton myasthenic syndrome. A review of 50 cases. *Brain* 1988; **111** (Pt 3): 577-596 [PMID: 2838124]
  - 37 **Titulaer MJ**, Soffietti R, Dalmau J, Gilhus NE, Giometto B, Graus F, Grisold W, Honnorat J, Sillevius Smitt PA, Tanasescu R, Vedeler CA, Voltz R, Verschuuren JJ. Screening for tumours in paraneoplastic syndromes: report of an EFNS task force. *Eur J Neurol* 2011; **18**: 19-e3 [PMID: 20880069]
  - 38 **Keogh M**, Sedehizadeh S, Maddison P. Treatment for Lambert-Eaton myasthenic syndrome. *Cochrane Database Syst Rev* 2011; CD003279 [PMID: 21328260]
  - 39 **Molgó J**, Lundh H, Thesleff S. Potency of 3,4-diaminopyridine and 4-aminopyridine on mammalian neuromuscular transmission and the effect of pH changes. *Eur J Pharmacol* 1980; **61**: 25-34 [PMID: 6101553]
  - 40 **Lindquist S**, Stangel M. Update on treatment options for Lambert-Eaton myasthenic syndrome: focus on use of amifampridine. *Neuropsychiatr Dis Treat* 2011; **7**: 341-349 [PMID: 21822385]
  - 41 **Sanders DB**, Massey JM, Sanders LL, Edwards LJ. A randomized trial of 3,4-diaminopyridine in Lambert-Eaton myasthenic syndrome. *Neurology* 2000; **54**: 603-607 [PMID: 10680790]
  - 42 **Skeie GO**, Apostolski S, Evoli A, Gilhus NE, Illa I, Harms L, Hilton-Jones D, Melms A, Verschuuren J, Horge HW. Guidelines for treatment of autoimmune neuromuscular transmission disorders. *Eur J Neurol* 2010; **17**: 893-902 [PMID: 20402760]
  - 43 **Maddison P**, McConville J, Farrugia ME, Davies N, Rose M, Norwood F, Jungbluth H, Robb S, Hilton-Jones D. The use of rituximab in myasthenia gravis and Lambert-Eaton myasthenic syndrome. *J Neurol Neurosurg Psychiatry* 2011; **82**: 671-673 [PMID: 20392977]
  - 44 **Elovaara I**, Apostolski S, van Doorn P, Gilhus NE, Hietaharju A, Honkaniemi J, van Schaik IN, Scolding N, Soelberg Sørensen P, Udd B. EFNS guidelines for the use of intravenous immunoglobulin in treatment of neurological diseases: EFNS task force on the use of intravenous immunoglobulin in treatment of neurological diseases. *Eur J Neurol* 2008; **15**: 893-908 [PMID: 18796075]
  - 45 **Reuner U**, Kamin G, Ramantani G, Reichmann H, Dinger J. Transient neonatal Lambert-Eaton syndrome. *J Neurol* 2008; **255**: 1827-1828 [PMID: 18758885]
  - 46 **Weimer MB**, Wong J. Lambert-eaton myasthenic syndrome. *Curr Treat Options Neurol* 2009; **11**: 77-84 [PMID: 19210909]
  - 47 **Wirtz PW**, Willcox N, van der Slik AR, Lang B, Maddison P, Koeleman BP, Giphart MJ, Wintzen AR, Roep BO, Verschuuren JJ. HLA and smoking in prediction and prognosis of small cell lung cancer in autoimmune Lambert-Eaton myasthenic syndrome. *J Neuroimmunol* 2005; **159**: 230-237 [PMID: 15652424]
  - 48 **Ko MW**, Dalmau J, Galetta SL. Neuro-ophthalmologic manifestations of paraneoplastic syndromes. *J Neuroophthalmol* 2008; **28**: 58-68 [PMID: 18347462]
  - 49 **Brain L**, Wilkinson M. Subacute cerebellar degeneration associated with neoplasms. *Brain* 1965; **88**: 465-478 [PMID: 5890520]
  - 50 **Dalmau J**, Rosenfeld MR. Paraneoplastic syndromes of the CNS. *Lancet Neurol* 2008; **7**: 327-340 [PMID: 18339348]
  - 51 **Fukuda T**, Motomura M, Nakao Y, Shiraishi H, Yoshimura T, Iwanaga K, Tsujihata M, Eguchi K. Reduction of P/Q-type calcium channels in the postmortem cerebellum of paraneoplastic cerebellar degeneration with Lambert-Eaton myasthenic syndrome. *Ann Neurol* 2003; **53**: 21-28 [PMID: 12509844]
  - 52 **Martín-García E**, Mannara F, Gutiérrez-Cuesta J, Sabater L, Dalmau J, Maldonado R, Graus F. Intrathecal injection of P/Q type voltage-gated calcium channel antibodies from paraneoplastic cerebellar degeneration cause ataxia in mice. *J Neuroimmunol* 2013; **261**: 53-59 [PMID: 23726906]
  - 53 **Choi KD**, Kim JS, Park SH, Kim SE, Smitt PS. Cerebellar hypermetabolism in paraneoplastic cerebellar degeneration. *J Neurol Neurosurg Psychiatry* 2006; **77**: 525-528 [PMID: 16543536]
  - 54 **Bürk K**, Wick M, Roth G, Decker P, Voltz R. Antineuronal antibodies in sporadic late-onset cerebellar ataxia. *J Neurol* 2010; **257**: 59-62 [PMID: 19629562]
  - 55 **Lorenzoni PJ**, Scola RH, Lang B, Kay CS, Teive HA, Kowacs PA, Werneck LC. Cerebellar ataxia in non-paraneoplastic Lambert-Eaton myasthenic syndrome. *J Neurol Sci* 2008; **270**: 194-196 [PMID: 18374949]



- 56 **Pellkofer HL**, Voltz R, Kuempfel T. Favorable response to rituximab in a patient with anti-VGCC-positive Lambert-Eaton myasthenic syndrome and cerebellar dysfunction. *Muscle Nerve* 2009; **40**: 305-308 [PMID: 19609921]
- 57 **Rigamonti A**, Lauria G, Stanzani L, Mantero V, Andreetta F, Salmaggi A. Non-paraneoplastic voltage-gated calcium channels antibody-mediated cerebellar ataxia responsive to IVIG treatment. *J Neurol Sci* 2014; **336**: 169-170 [PMID: 24215945]

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## Evaluation of functional, autonomic and inflammatory outcomes in children with asthma

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throes of an attack. Chronic inflammation is caused by an imbalance between pro-inflammatory and anti-inflammatory mechanisms as well as autonomic dysfunction, which plays an important role in the pathogenesis and control of this condition. The impact of these physiopathological aspects leads to inactivity and a sedentary lifestyle, which exerts an influence on functional capacity and control of the disease. The main objective of non-pharmacological therapy is the clinical control of asthma and the minimization of airway obstruction and hyperinflation during an attack. These factors can be controlled with noninvasive ventilation. The aim of the present review was to describe important neural, inflammatory and functional mechanisms that affect children with asthma.

**Key words:** Asthma; Child; Continuous positive airway pressure; Noninvasive ventilation; Autonomic nervous system; Functional capacity; Inflammatory mechanisms; Evaluation

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**Core tip:** The recurring nature of asthma is related to the clinical control of the disease. Neural and inflammatory mechanisms interfere with this clinical control and affect functional capacity. While the magnitude of an asthma attack cannot be controlled, its clinical impact can be minimized with the use of noninvasive ventilation. Moreover, functional capacity and inflammation can be improved with physical exercise.

### Abstract

Asthma is common in childhood. This respiratory disease is characterized by persistent inflammation of the airways even when the child is not in the

de Freitas Dantas Gomes EL, Costa D. Evaluation of functional, autonomic and inflammatory outcomes in children with asthma. *World J Clin Cases* 2015; 3(3): 301-309 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v3/i3/301.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v3.i3.301>

## INTRODUCTION

Asthma is common in childhood. This respiratory disease is characterized by persistent inflammation of the airways, which is clinically manifested in the form of recurring cough, shortness of breath, wheezing and chest retraction. These episodes are associated with the obstruction of airflow, which is partially reversible and occurs mainly in the morning and at night<sup>[1,2]</sup>.

Controlling the disease is the main objective of asthma management. Such control refers to the most recent clinical manifestations (previous four weeks) with regard to symptoms, limitations to physical activity, the need for a  $\beta_2$  agonist and the intensity of airflow limitation as well as the reduction of future risks. Asthma is therefore classified as controlled, partially controlled or uncontrolled. Addressing future risks regards reducing the instability of asthma and exacerbations, the loss of lung function and the adverse effects of treatment<sup>[2,3]</sup>.

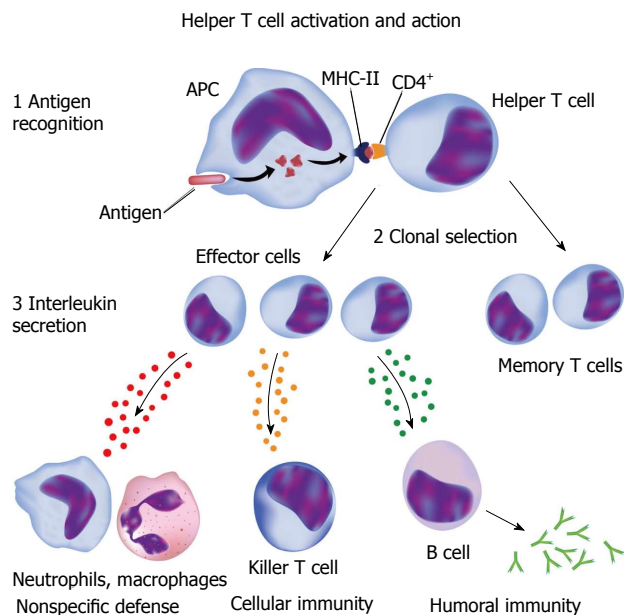
The incidence of asthma has doubled in the last 20 years due to the actual increase in the number of cases as well as better recognition of the disease on the part of the medical community. The difficulty in comparing epidemiological data from different countries or even different regions within the same country motivated the International Study of Asthma and Allergies in Childhood, which employed a simple, validated, self-administered questionnaire with a small number of items. The authors of the study evaluated 304796 children aged six to seven years from 42 countries and 463801 adolescents aged 13 to 14 years at 155 centers in 56 countries and distinguished three categories of countries based on the prevalence rates of asthma: weak (less than 5%), medium (5% to 6%) and strong (greater than 10%). Brazil was classified in eight place, with a prevalence rate of 20%<sup>[4,5]</sup>.

Despite the difficulties in diagnosing asthma in children, there is evidence that half of all cases are diagnosed by three years of age and 80% are diagnosed by six years of age. Moreover, one third of the initial symptoms begin before the child has completed one year of life. Although 50% of children with asthma in Latin America exhibit daily symptoms and arousals from sleep, only 10% regularly use medication to control asthma and only treat attacks with an inhaler, while only 13% employ preventive measures and the control of exacerbations<sup>[6]</sup>.

The aim of the present review was to describe important neural, inflammatory and functional mechanisms that affect children with asthma.

## PHYSIOPATHOLOGY AND INFLAMMATION IN ASTHMA

Asthma is an inflammatory disease involving the participation of mastocytes, eosinophils, T cells and dendritic cells. Among the different phenotypes, atopic

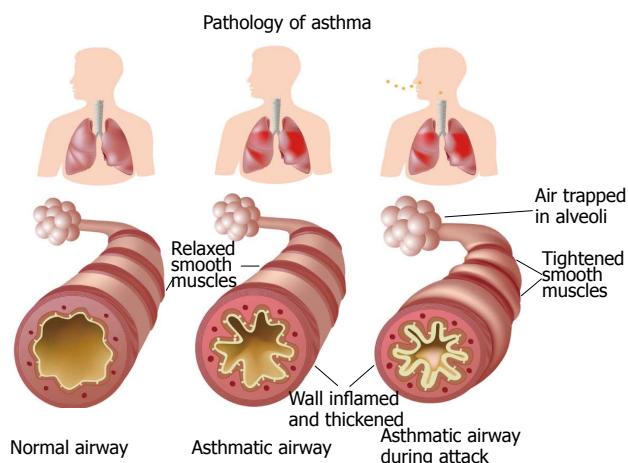


**Figure 1** Cellular and humoral response to an antigen. © Can Stock Photo Inc./[Alila].

asthma is the most common and is characterized by an increase in eosinophils and total immunoglobulin E (IgE), with the considerable participation of mastocytes and their products in events during the acute phase. These cells also participate in the chronic inflammatory process and bronchial hyperresponsiveness, along with macrophages, eosinophils and T lymphocytes<sup>[2]</sup>. Macrophages can either increase or diminish the inflammatory process, depending on the stimulus. Alveolar macrophages normally suppress lymphocyte function, but this function may be altered in individuals with asthma following exposure to a triggering factor<sup>[7]</sup> (Figure 1).

Eosinophil infiltrate is a physiopathological characteristic of the airways in individuals with asthma and contributes to the differentiation of this disease from other inflammatory conditions. Eosinophils are seen as beneficial in asthma due to their function in inactivating histamine and leukotrienes. However, eosinophils are also involved in injurious processes of the airways tissues, contributing to the development of bronchial hyperresponsiveness.

Chronic inflammation results in the obstruction of distal airways due to the accumulation of secretion and cell debris, the contraction of bronchial smooth muscles, thickening of the epithelial basement membrane and bronchial wall edema<sup>[8]</sup>. The most striking characteristic of asthma is persistent inflammation of the airways even when the child is not in the throes of an attack. The degree of inflammation is associated with bronchial hyperresponsiveness and symptoms. Chronic inflammation is caused by an imbalance between pro-inflammatory and anti-inflammatory mechanisms<sup>[9]</sup>. The persistence of this inflammatory condition over the years combined with frequent acute attacks can exert a



**Figure 2** Bronchi in normal conditions, asthma and during asthma attack. © Can Stock Photo Inc./[Alila].

negative influence on lung function (Figure 2).

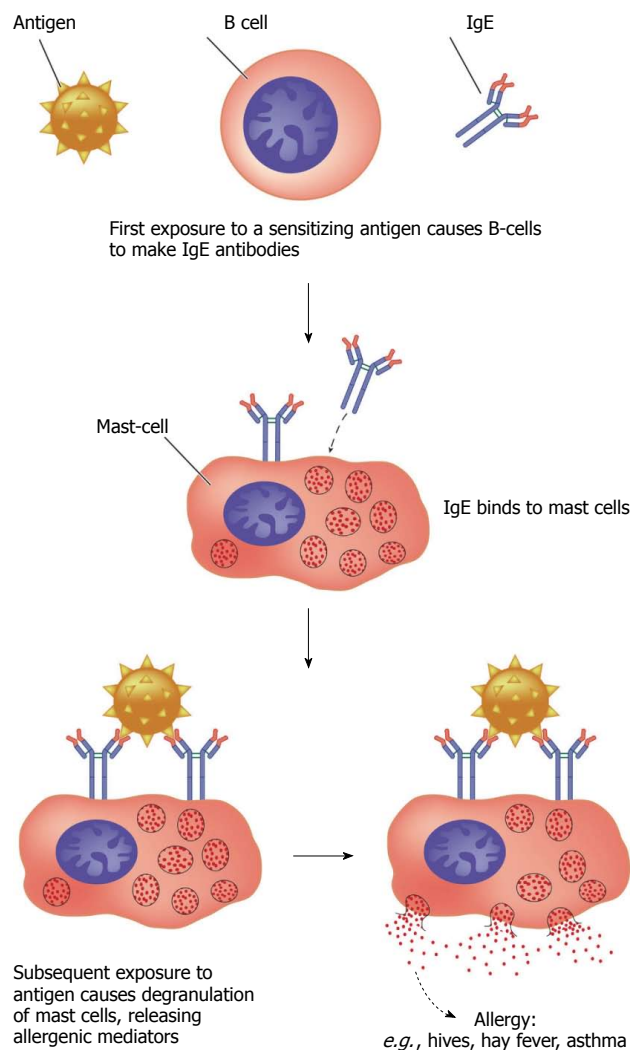
Inflammation has been the focus of treatment for asthma in the last 20 years. Prior to the 1980s, asthma was seen as recurrent wheezing that responded to  $\beta_2$  agonists. The inflammatory reaction begins with contact between an allergen (pollutant or virus) and dendritic cells, which activate mastocytes that, in turn, release IgE specific to given allergens. This initial process leads to the release of different mediators. The consequence of this initial phase (four to six hours) of the inflammatory cascade is clinically manifested as bronchospasms<sup>[10]</sup>. In the late phase of the inflammatory reaction, granulocytes are released by bone marrow and migrate to the target organ (lungs), causing tissue injury and the release of toxins<sup>[11,12]</sup>. Inflammatory cytokines stimulate the formation of inducible nitric oxide synthase (iNOS). At high concentrations, NO is the major contributor to the inflammatory process in asthma<sup>[13]</sup> (Figure 3).

Cell apoptosis is maintained activated at all moments of the inflammatory cascade, possibly reducing the reserve of cells that could cease this process. Asthma is characterized by a reduction in apoptotic potential and consequent perpetuation of the inflammatory process. Moreover, the molecules involved in the tissue repair process seem to be relatively ineffective. These events results in chronic inflammation and the structural remodeling of the airways. All these factors seem to be the basis for bronchial hyperreactiveness and other symptoms<sup>[10-12]</sup>.

#### Differences between childhood and adult asthma

Childhood is the period of greatest incidence of asthma, however the identification of these children still problematic. The clinical syndrome that is recognized as asthma fails to develop in all children and infants with wheezing (in less than half as already stated earlier).

Another difficulty in the identification of asthma in childhood is related to pulmonary function test as



**Figure 3** Activation of mast cells and release of IgE. © Can Stock Photo Inc./[Blambs].

well as the documentation of the obstruction and its reversibility objectively and quantitatively in small children. The challenge is to detect by means of non-invasive biomarkers in infants and pre school children who can become asthmatic by facilitating in this way the clinical control and reducing the morbidity of the disease in adulthood.

What is known so far that insults occurred postnatally as aspiration, viral infections or allergic sensitization may alter the airway neural control for long periods<sup>[13]</sup>.

#### Respiratory infection and asthma in childhood

Various pathogens may cause wheezing in childhood, in particular, respiratory syncytial virus (RSV). Bronchiolitis from RSV increases the risk for recurrent wheezing (more than 3 episodes of wheezing per year) and not persistent (up to 3 episodes of wheezing per year); However, the risk gradually decreases with age and becomes not significant to 13 years of age<sup>[14]</sup>. These data indicate that RSV infections may contribute substantially to the risk of recurrent wheezing and possibly asthma in childhood and also suggest that



other co-factors (for example: genetic, environmental and development) contribute to the onset or severity of asthma over time.

Respiratory infections and asthma are closely related and children are very susceptible to these infections, but less than half of them develop recurrent wheezing.

Some types of respiratory infection in infancy can stimulate Th1 cells and as regards the pathophysiology of childhood asthma in the balance and the way in which this balance Th1/Th2 is reached in this age group still generate doubts. What is known is that the non atopic child have a reduction of Th2 in the first year of life, already in atopic child Th2 response is associated with the production of IFN- $\gamma$  in the neonatal period<sup>[14]</sup>.

Atopic asthma is characterized by this imbalance Th1/Th2. Th2 cells when stimulated produces interleukin 4 (IL4) and IL13 that induce the production of IgE by B cells. The IL9 is also produced by Th2 stimulates the proliferation of mast cells which in turn starts the production of histamine, leukotrienes and prostaglandins that lead to an additional activation of eosinophils, basophils and Th2<sup>[15]</sup>.

The endothelial lesion caused by viral infection leads to increased airway permeability facilitating the exposure of allergens to cells in the nerve endings promoting a neurogenic inflammation. Severe respiratory infections increase the recruitment of eosinophils to the airways bringing the risk of persistent asthma in childhood<sup>[13]</sup>.

### **Fraction of exhaled nitric oxide**

Gustafsson *et al.*<sup>[16]</sup> (1991) were the first researchers to isolate the nitric oxide (NO) molecule. According to the authors, NO is formed by the action of NO synthase on the semi-essential amino acid L-arginine. Two basic isoforms are produced: constitutive and inducible<sup>[17]</sup>. NO synthase performs a number of biological functions, such as neurotransmission, vasodilatation and bronchodilatation as well as playing a role in the immune system. The type I constitutive isoform (epithelial) promotes vasodilatation and the type II constitutive isoform (neuronal) is responsible for the transmission of stimuli from the central nervous system and autonomic nervous system through the non-adrenergic, non-cholinergic pathway<sup>[17,18]</sup>. The inducible isoform (iNOS) is stimulated with the perpetuation of the inflammatory process and contributes to this process due to its pro-inflammatory activity. When activated, this isoform promotes an increase in the production of secretion in the airways and a large number of inflammatory cells, favoring necrosis of the ciliated tissue<sup>[7,19]</sup>. The constitutive isoforms are produced in small amounts that are not detectable in exhaled breath and perform their biological and physiological roles, whereas iNOS is produced in large amounts, has cytotoxic effects and is detectable in exhaled breath (FeNO)<sup>[20,21]</sup>.

## **FUNCTIONAL CAPACITY**

Fear of shortness of breath often makes children with asthma avoid physical exercise, leading to a sedentary lifestyle, psychological and postural problems as well as a poor quality of life. Studies have demonstrated that children with moderate to severe uncontrolled asthma exhibit chronic physical deconditioning and reduced cardiopulmonary capacity<sup>[22,23]</sup>. Villa *et al.*<sup>[24]</sup> (2011) evaluated children with persistent moderate to severe asthma and found that those with severe asthma exhibited diminished lower limb endurance. Thus, controlled physical activities are needed to prevent the impairment of cardiopulmonary capacity and physical fitness.

Repeated physical activities with varying intensity that last only a few seconds and are intercalated with short rest periods are more appropriate for children. Besides preferring spontaneous activities with a recreational components and considerable variety, children explore the anabolic effects of physical exercise more<sup>[25]</sup>. Thus, physically-based play activities are indicated for children with asthma. While 40% to 90% of children with asthma exhibited exercise-induced bronchospasms, the regular practice of physical activity is able to improve this symptom, often with no direct impact on lung function<sup>[2]</sup>.

Advances in technology have facilitated the performance of movements and physical activity<sup>[26-28]</sup>. Interactive video games in the last ten years have contributed to energy expenditure among otherwise sedentary children, with the possible applications regarding lung rehabilitation among children with asthma. However, previous studies have only employed this resource for the training of children without lung diseases<sup>[29]</sup>. Thus, there is a need for scientific evidence of the benefits of interactive video games for the improvement of physical fitness in this population.

### **Inflammation and physical exercise**

A persistent inflammatory state is a common trait of chronic diseases. Inflammation is indicated by the high concentration of inflammatory markers, such as IL6, tumor necrosis factor alpha (TNF $\alpha$ ) and C-reactive protein in the blood plasma<sup>[30]</sup>. Physical exercise has an anti-inflammatory effect and regular practice seem to offer protection against the development and aggravation of chronic diseases. While children with asthma tend to avoid physical exertion, the practice of physical exercise can be beneficial to this population. However, there are no specific guidelines with regard to the type, intensity, duration and frequency of training<sup>[31]</sup>.

Three mechanisms seem to explain the anti-inflammatory effect of physical exercise. The first is the reduction in visceral fat, as excess fat contributes to the production of pro-inflammatory adipokines,

such as TNF and leptin, as well as the reduction in adiponectin. The second mechanism is the increase in the production and release of anti-inflammatory cytokines, such as IL6, stemming from muscle contractions caused by myosin, which induces different metabolic effects, such as lipolysis and the oxidation of fat, as well as contributing to the homeostasis of glucose during physical exercise. The third mechanism is the reduction in the expression of receptors of monocytes and macrophages, which have a lower inflammatory response to endotoxins in physically active individuals<sup>[30-33]</sup>.

The effect of myokines (IL6 released by the contraction of skeletal muscle) regulates the release of TNF $\alpha$ , resulting in a protective effect<sup>[34]</sup> (Tilg *et al.*<sup>[34]</sup> 1997). Moreover, IL6 stimulates the release of the anti-inflammatory interleukins 10 and 1ra. IL10 inhibits the production of interleukins 1a and 1b as well as the induction of iNOS<sup>[35]</sup>. This mechanism may explain the likely effect of physical exercise on changes in the concentration of FeNO.

## AUTONOMIC NERVOUS SYSTEM AND CONNECTIONS WITH RESPIRATORY AND CARDIOVASCULAR SYSTEMS

The respiratory and cardiovascular systems are intrinsically linked and the autonomic nervous system (ANS) is one of the pathways that connect these systems<sup>[36]</sup> (Figure 4).

Moreover, the ANS exerts an important influence on the pathogenesis and control of asthma<sup>[37]</sup>. Afferent pulmonary nerve pathways regulate cholinergic tone, which is increased when the respiratory rate is increased. This mechanism occurs in response to physiological and physiopathological stimuli. A physiological response occurs when the peripheral respiratory muscles and pulmonary stretch receptors send afferent stimuli to the central nervous system during physical exertion, which results in the reduction in cholinergic tone as well as bronchial dilatation to compensate for the increase in ventilation demand. This mechanism is altered in individuals with asthma<sup>[37]</sup>.

Understanding the relationships between the ANS and respiratory system may help clarify the causes of cardiovascular disorders with a pulmonary origin. From the neuroanatomic standpoint, afferent and efferent neural pathways of the respiratory and cardiovascular systems converge in common regions. Afferent pathways converge in the solitary tract nucleus and efferent pathways converge in the nucleus ambiguus, which is responsible for the generation of the respiratory rate and heart rate<sup>[36]</sup>.

In individuals with asthma, the inflammatory process is increased during periods of exacerbation. The increase in vagal tone during stable periods of the disease may be explained by an attempt to maintain inflammatory control. The ANS exerts an influence on

the relaxation and constriction of the smooth muscles of the bronchioles. Relaxation occurs through either the activation of beta receptors of the sympathetic pathway or the activation of the non-adrenergic, non-cholinergic and intestinal peptide pathways. Constriction is mediated by either sympathetic receptors or the vagal cholinergic pathway<sup>[37]</sup>.

Heart rate variability has been used as a measure of vagal autonomic activity and neuroimmunomodulation. A number of studies have addressed the influence of respiration on heart rate variability. Chronic respiratory diseases, such as asthma, demonstrate a link between the inflammatory process and the immune reaction as well as progression with cardiovascular consequences that can contribute to the increase in illness and mortality rates<sup>[36]</sup>.

### Asthma and autonomic modulation

The activation of the autonomic mechanism in the respiratory system is due to the reflex response of receptors located in the airways, regulating bronchial contractility<sup>[38-41]</sup>. Autonomic dysfunction is associated with asthma<sup>[42,43]</sup>, with an increase in bronchial sensitivity to cholinergic constrictors and possibly a reduction in sensitivity to adrenergic  $\beta_2$  bronchodilators. Besides its essential role in the cardiovascular system, the ANS plays an important role in the regulation of the contraction of bronchial smooth muscles<sup>[37]</sup>.

Asthma severity is directed related to autonomic dysfunction even under conditions in which the patient is not a period of exacerbation. The functions of the ANS in children with asthma also differ in comparison to adults and healthy children<sup>[43,44]</sup>. This severity is correlated with the magnitude of vagal activation in the stable phase<sup>[43]</sup>. During periods of exacerbation, children with persistent moderate asthma experience greater action of the sympathetic nervous system, unlike what occurs in stable periods, which conflicts with the hypothesis of hyperactivity of vagal tone in the acute phase<sup>[45]</sup>.

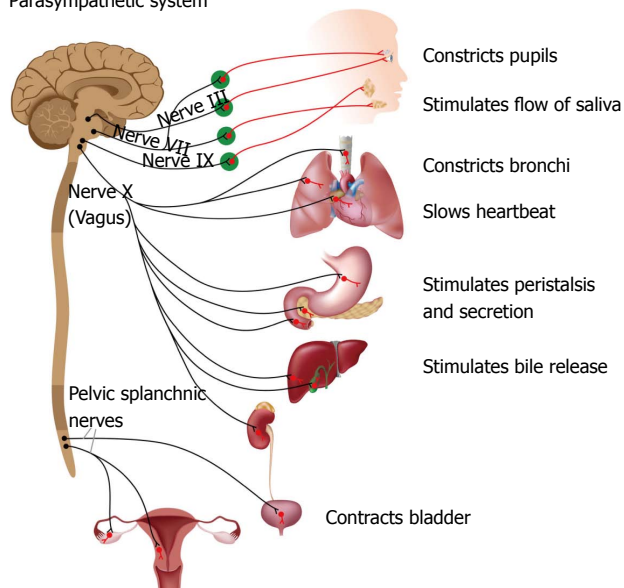
Neural mechanisms are also responsible for the regulation of inflammation. The activation of the vagus nerve is responsible for the inhibition of the activation of macrophages and the synthesis of TNF $\alpha$  as well as the activation of the reticuloendothelial system for the release of acetylcholine<sup>[46]</sup>.

### Autonomic modulation and degree of effort in asthma

As mentioned above, asthma progresses with autonomic abnormality during physical effort. The ANS responds to stimuli sent by the muscles, lungs and diaphragm for the withdrawal of vagal tone, resulting in an increase in the diameter of the airways. This is a mechanical response to a neural stimulus that allows enhancing the airflow, with a consequent improvement in gas exchange in response to the increase in metabolic demand<sup>[37]</sup>.

Different degrees of physical stress can trigger changes in autonomic modulation in children with

## Parasympathetic system



## Parasympathetic system

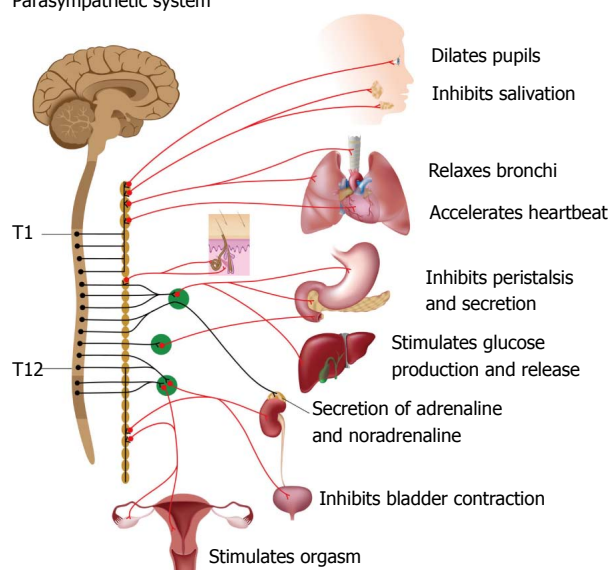


Figure 4 Autonomic nervous system subdivision. © Can Stock Photo Inc./[Alila].

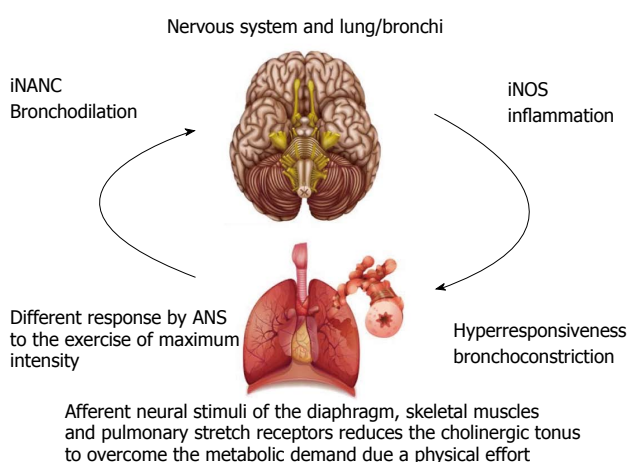


Figure 5 Lung and nervous system interaction. © Can Stock Photo Inc. / [Alexilus and Bluering].

asthma. During physical exertion, the heart rate and cardiac output are increased mainly due to vagal withdrawal stemming from a central command. In the transition from an intense activity involving an increase in heart rate greater than 100 beats per minute, the increase in sympathetic activity is necessary to induce tachycardia as well as increase both heart contractibility and peripheral vascular resistance<sup>[47]</sup>. During maximum physical effort, this mechanism of vagal withdrawal and sympathetic activation seems to be altered and relatively ineffective in children with asthma. Vagal withdrawal also seems to occur during sub-maximum effort<sup>[48]</sup> (Figure 5).

### Management of shortness of breath and respiratory failure using noninvasive ventilation

During respiratory distress, children with asthma

experience bronchial obstruction and hyperinflation, which predisposes such individuals to respiratory failure. Thus, the aims of the management of severe acute asthma include the correction of hypoxemia, alleviation of the airflow obstruction and the reduction of the inflammatory process through medications. The therapeutic goal is to reduce respiratory work and optimize gas exchange. Continuous positive airway pressure (CPAP), which is a form of noninvasive ventilation (NIV), is used to reduce the work of respiratory muscles imposed by hyperinflation of the lungs and produces a change in autonomic modulation stemming from the increase in intra-thoracic pressure<sup>[49,50]</sup> (Figure 6).

There is scientific evidence of the efficacy of NIV for an asthma attack. Gupta *et al.*<sup>[51]</sup> (2010) report improved respiratory work, fast recovery of lung function as well as reductions in inhaler dose, stay in an intensive care unit and the duration of hospitalization among adults with acute asthma treated with bi-level NIV. CPAP caused changes in both alveolar and intra-thoracic pressures and the activation of pulmonary stretch receptors affects autonomic modulation<sup>[37,50]</sup>. In a study involving an experimental model, Xue *et al.*<sup>[52]</sup> (2011) found that the increase in intra-thoracic pressure leads to a reduction in bronchial hyperresponsiveness, which persists for up to 24 h following the removal of CPAP. Busk *et al.*<sup>[53]</sup> (2013) found a reduction in bronchial hyperresponsiveness in adults with asthma after seven days of treatment and consider CPAP to be an important non-pharmacological tool for the treatment of this condition.

Parasympathetic nerve stimulation results in both the contraction and relaxation of the bronchial musculature. The non-adrenergic, non-cholinergic

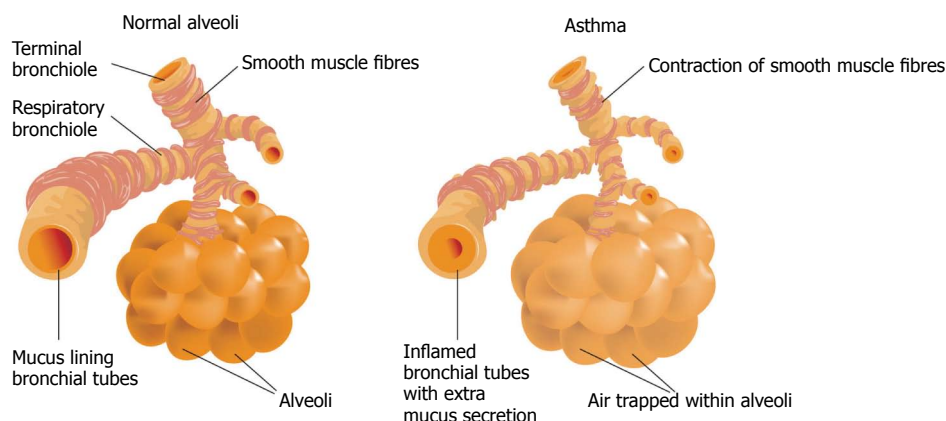


Figure 6 Hyperinflation. © Can Stock Photo Inc. / [Blambs].

pathway (bronchodilatation) is stimulated by pulmonary stretch receptors. Contraction is mediated by acetylcholine and relaxation is mediated by transmitters of the non-adrenergic, non-cholinergic pathway. Cholinergic tone is extremely sensitive to the ventilatory mechanism and is reduced when the respiratory rate is reduced<sup>[37]</sup>. Thus, CPAP may achieve its positive effects by reducing the respiratory rate and stimulating the non-adrenergic, non-cholinergic pathway through pulmonary stretch receptors. The acute effects of CPAP on autonomic modulation have been demonstrated in other pathological conditions, but there is a lack of scientific evidence regarding the benefits of this method in patients (especially children) with asthma<sup>[50]</sup>.

In a study conducted by our research group<sup>[54]</sup> involving the evaluation of clinical variables and the ANS in children in the throes of an asthma attack, the activation of vagal tone was found with the administration of CPAP. Parasympathetic activation stimulates both contraction and relaxation of the smooth muscles of the airways. Cholinergic tone is reduced with positive end-expiratory pressure and the reduction in the respiratory rate<sup>[37,55]</sup>.

The findings suggest that the non-cholinergic parasympathetic pathway is activated by the administration of CPAP due to the significant increase in peak flow, which continues after the removal of NIV, suggesting a bronchodilatation response as well as the mechanical stimulation of the opening of the airways. Pulmonary stretch and the activation of the non-cholinergic pathway is believed to assist in the inhibition of the cholinergic bronchoconstriction pathway<sup>[37]</sup>.

## CONCLUSION

The recurrent nature of asthma is related to the clinical control of the disease. Neural and inflammatory mechanisms interfere with this control and affect functional capacity. While the magnitude of an asthma attack cannot be controlled, its clinical impact can be minimized with the use of noninvasive ventilation. Moreover, functional capacity and inflammation can be

improved with physical exercise.

## REFERENCES

- 1 McCormack MC, Enright PL. Making the diagnosis of asthma. *Respir Care* 2008; **53**: 583-90; discussion 590-2 [PMID: 18426612]
- 2 Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2011. Available from: URL: <http://www.ginasthma.org>
- 3 Diretrizes da SBPT no manejo da asma. *J Bras Pneumol* 2012; **38** (supl 1): S1-S46
- 4 Asher MI, Weiland SK. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC) *Eur Respir J* 1998; **12**: 315-335 [PMID: 9727780 DOI: 10.1183/09031936.98.12020315]
- 5 Patt JA, Eberhart RJ. Effects of metyrapone and ACTH on intestinal absorption of immunoreactive bovine IgG in cesarean-derived pigs. *Am J Vet Res* 1976; **37**: 1409-1413 [PMID: 187090]
- 6 Neffen H, Fritscher C, Schacht FC, Levy G, Chiarella P, Soriano JB, Mechali D. Asthma control in Latin America: the Asthma Insights and Reality in Latin America (AIRLA) survey. *Rev Panam Salud Publica* 2005; **17**: 191-197 [PMID: 15826399]
- 7 Barnes PJ. Pathophysiology of asthma. *Br J Clin Pharmacol* 1996; **42**: 3-10 [PMID: 8807137]
- 8 Payne D, Bush A. Phenotype-specific treatment of difficult asthma in children. *Paediatr Respir Rev* 2004; **5**: 116-123 [PMID: 15135121 DOI: 10.1016/j.prrv.2004.01.006]
- 9 Dodig S, Richter D, Zrinskotopic R. Inflammatory markers in childhood asthma. *Clin Chem Lab Med* 2011; **49**: 587-599 [PMID: 21303302 DOI: 10.1515/CCLM.2011.094]
- 10 Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000; **161**: 1720-1745 [PMID: 10806180 DOI: 10.1164/ajrccm.161.5.9903102]
- 11 Papadopoulos NG, Xepapadaki P, Mallia P, Brusselle G, Watelet JB, Xatzipsalti M, Foteinos G, van Drunen CM, Fokkens WJ, D'Ambrosio C, Bonini S, Bossios A, Lötvall J, van Cauwenberge P, Holgate ST, Canonica GW, Szczeklik A, Rohde G, Kimpen J, Pitkäranta A, Mäkelä M, Chanez P, Ring J, Johnston SL. Mechanisms of virus-induced asthma exacerbations: state-of-the-art. A GA2LEN and InterAirways document. *Allergy* 2007; **62**: 457-470 [PMID: 17324199 DOI: 10.1111/j.1398-9995.2007.01341.x]
- 12 Denburg JA, Inman MD, Leber B, Sehmi R, O'Byrne PM. The role of the bone marrow in allergy and asthma. *Allergy* 1996; **51**: 141-148 [PMID: 8781667]
- 13 Larsen GL. Differences between adult and childhood asthma. *J Allergy Clin Immunol* 2000; **106**: S153-S157 [PMID: 10984396]
- 14 Oh JW. Respiratory viral infections and early asthma in childhood. *Allergol Int* 2006; **55**: 369-372 [PMID: 17130678 DOI: 10.2332/



- allergolint.55.369]
- 15 **Vijverberg SJ**, Hilvering B, Raaijmakers JA, Lammers JW, Maitland-van der Zee AH, Koenderman L. Clinical utility of asthma biomarkers: from bench to bedside. *Biologics* 2013; **7**: 199-210 [PMID: 24009412 DOI: 10.2147/BTT.S29976]
  - 16 **Gustafsson LE**, Leone AM, Persson MG, Wiklund NP, Moncada S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem Biophys Res Commun* 1991; **181**: 852-857 [PMID: 1721811 DOI: 10.1016/0006-291X(91)91268-H]
  - 17 **Chatkin JM**, Djupesland P, Qian W, Haight J, Zamel N. Óxido Nítrico exalado no diagnóstico e acompanhamento das doenças respiratórias. *J Pneumol* 2000; **26**: 36-43 [DOI: 10.1590/S0102-35862000000100008]
  - 18 **Manna A**, Caffarelli C, Varini M, Povesi Dascola C, Montella S, Maglione M, Sperli F, Santamaria F. Clinical application of exhaled nitric oxide measurement in pediatric lung diseases. *Ital J Pediatr* 2012; **38**: 74 [PMID: 23273317 DOI: 10.1186/1824-7288-38-74]
  - 19 **Guembe L**, Villaro AC. Histochemical demonstration of neuronal nitric oxide synthase during development of mouse respiratory tract. *Am J Respir Cell Mol Biol* 1999; **20**: 342-351 [PMID: 9922227 DOI: 10.1165/ajrcmb.20.2.3319]
  - 20 **Eynott PR**, Groneberg DA, Caramori G, Adcock IM, Donnelly LE, Kharitonov S, Barnes PJ, Chung KF. Role of nitric oxide in allergic inflammation and bronchial hyperresponsiveness. *Eur J Pharmacol* 2002; **452**: 123-133 [PMID: 12323393 DOI: 10.1016/S0014-2999(02)02237-9]
  - 21 **Kobayashi H**, Cui T, Ando M, Hataishi R, Imasaki T, Mitsufuji H, Hayashi I, Tomita T. Nitric oxide released from iNOS in polymorphonuclear leukocytes makes them deformable in an autocrine manner. *Nitric Oxide* 2002; **7**: 221-227 [PMID: 12381419 DOI: 10.1016/S1089-8603(02)00109-X]
  - 22 **Vahlkvist S**, Pedersen S. Fitness, daily activity and body composition in children with newly diagnosed, untreated asthma. *Allergy* 2009; **64**: 1649-1655 [PMID: 19489758 DOI: 10.1111/j.1398-9995.2009.02081.x]
  - 23 **Green RJ**, Klein M, Becker P, Halkas A, Lewis H, Kitchin O, Moodley T, Masekela R. Disagreement among common measures of asthma control in children. *Chest* 2013; **143**: 117-122 [PMID: 22878380 DOI: 10.1378/chest.12-1070]
  - 24 **Villa F**, Castro AP, Pastorino AC, Santarém JM, Martins MA, Jacob CM, Carvalho CR. Aerobic capacity and skeletal muscle function in children with asthma. *Arch Dis Child* 2011; **96**: 554-559 [PMID: 21429976 DOI: 10.1136/adc.2011.212431]
  - 25 **Zwiren LD**. Considerações sobre testes de esforço e sua prescrição durante a infância in Manual de Pesquisa das diretrizes do ACSM (American College Sports medicine) para os testes de esforço e sua prescrição. Guanabara Koogan 4ª Edição, 2003: 522-528
  - 26 **Wanrooij VH**, Willeboordse M, Dompeling E, van de Kant KD. Exercise training in children with asthma: a systematic review. *Br J Sports Med* 2014; **48**: 1024-1031 [PMID: 23525551 DOI: 10.1136/bjsports-2012-091347]
  - 27 **Kuys SS**, Hall K, Peasey M, Wood M, Cobb R, Bell SC. Gaming console exercise and cycle or treadmill exercise provide similar cardiovascular demand in adults with cystic fibrosis: a randomised cross-over trial. *J Physiother* 2011; **57**: 35-40 [PMID: 21402328 DOI: 10.1016/S1836-9553(11)70005-4]
  - 28 **Graf DL**, Pratt LV, Hester CN, Short KR. Playing active video games increases energy expenditure in children. *Pediatrics* 2009; **124**: 534-540 [PMID: 19596737 DOI: 10.1542/peds.2008-2851]
  - 29 **Maddison R**, Mhurchu CN, Jull A, Prapavessis H, Foley LS, Jiang Y. Active video games: the mediating effect of aerobic fitness on body composition. *Int J Behav Nutr Phys Act* 2012; **9**: 54 [PMID: 22554052 DOI: 10.1186/1479-5868-9-54]
  - 30 **Gleeson M**, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol* 2011; **11**: 607-615 [PMID: 21818123 DOI: 10.1038/nri3041]
  - 31 **Pakhale S**, Luks V, Burkett A, Turner L. Effect of physical training on airway inflammation in bronchial asthma: a systematic review. *BMC Pulm Med* 2013; **13**: 38 [PMID: 23758826 DOI: 10.1186/1471-2466-13-38]
  - 32 **Pedersen BK**, Akerström TC, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. *J Appl Physiol* (1985) 2007; **103**: 1093-1098 [PMID: 17347387 DOI: 10.1152/japplphysiol.00080.2007]
  - 33 **Petersen AM**, Pedersen BK. The anti-inflammatory effect of exercise. *J Appl Physiol* (1985) 2005; **98**: 1154-1162 [PMID: 15772055 DOI: 10.1152/japplphysiol.00164.2004]
  - 34 **Tilg H**, Dinarello CA, Mier JW. IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. *Immunol Today* 1997; **18**: 428-432 [PMID: 9293158 DOI: 10.1016/S0167-5699(97)01103-1]
  - 35 **Cerqueira NF**, Yoshida WB. Óxido Nítrico - Revisão. *Acta Cir Bras* 2002; **17**: 417-423 [DOI: 10.1590/S0102-86502002000600011]
  - 36 **Thayer JF**, Loerbroeks A, Sternberg EM. Inflammation and cardiorespiratory control: the role of the vagus nerve. *Respir Physiol Neurobiol* 2011; **178**: 387-394 [PMID: 21642019 DOI: 10.1016/j.resp.2011.05.016]
  - 37 **Canning BJ**. Reflex regulation of airway smooth muscle tone. *J Appl Physiol* (1985) 2006; **101**: 971-985 [PMID: 16728519 DOI: 10.1152/japplphysiol.00313.2006]
  - 38 **Jammes Y**, Mei N. Assessment of the pulmonary origin of bronchoconstrictor vagal tone. *J Physiol* 1979; **291**: 305-316 [PMID: 480218]
  - 39 **Nadel JA**, Widdicombe JG. Reflex effects of upper airway irritation on total lung resistance and blood pressure. *J Appl Physiol* 1962; **17**: 861-865 [PMID: 13937041]
  - 40 **Spyer KM**. Central nervous integration of cardiovascular control. *J Exp Biol* 1982; **100**: 109-128 [PMID: 6294210]
  - 41 **Kollai M**, Jokkel G, Bonyhay I, Tomcsanyi J, Naszlady A. Relation between baroreflex sensitivity and cardiac vagal tone in humans. *Am J Physiol* 1994; **266**: H21-H27 [PMID: 8304501]
  - 42 **Fujii H**, Fukutomi O, Inoue R, Shinoda S, Okamoto H, Teramoto T, Kondo N, Wada H, Saito K, Matsuoka T, Seishima M. Autonomic regulation after exercise evidenced by spectral analysis of heart rate variability in asthmatic children. *Ann Allergy Asthma Immunol* 2000; **85**: 233-237 [PMID: 11030279 DOI: 10.1016/S1081-1206(10)62472-6]
  - 43 **Emin O**, Esra G, Aysegül D, Ufuk E, Ayhan S, Rusen DM. Autonomic nervous system dysfunction and their relationship with disease severity in children with atopic asthma. *Respir Physiol Neurobiol* 2012; **183**: 206-210 [PMID: 22789502 DOI: 10.1016/j.resp.2012.07.002]
  - 44 **Souza VF**, Costa IP, Silva AB, Lacerda DH, Costa D, Oliveira CS. Study of heart rate autonomic modulation in patients with asthma disease. *Med Scie Tech* 2010; **51**: 105-108
  - 45 **Gomes ELFD**, Sampaio LMM, Carvalho EFT, Mendes E, Peixoto-Souza FS, Costa D. Comparative analysis of autonomic modulation in children with acute and controlled asthma. *Med Scie Tech* 2013; **54**: 30-34 [DOI: 10.12659/MST.883863]
  - 46 **Thayer JF**. Vagal tone and the inflammatory reflex. *Cleve Clin J Med* 2009; **76** Suppl 2: S23-S26 [PMID: 19376977 DOI: 10.3949/ccjm.76.s2.05]
  - 47 **Rowell LB**. Human cardiovascular control Nova Iorque: Oxford University, 1993: 162-479
  - 48 **Gomes EL**, Sampaio LM, Costa IP, Dias FD, Férneda VS, Silva GA, Costa D. Analysis of autonomic modulation during maximal and submaximal work rate and functional capacity in asthmatic children. *J Asthma* 2013; **50**: 613-618 [PMID: 23574110 DOI: 10.3109/02770903.2013.793707]
  - 49 **Fessler HE**, Brower RG, Permutt S. CPAP reduces inspiratory work more than dyspnea during hyperinflation with intrinsic PEEP. *Chest* 1995; **108**: 432-440 [PMID: 7634880 DOI: 10.1378/chest.108.2.423]
  - 50 **Reis MS**, Sampaio LMM, Lacerda D, Oliveira LVF, Pereira GB, Pantoni CBF, Di Thommazo L, Catai AM, Borghi-Silva A. Acute Effects of different levels of continuous positive pressure on cardiac autonomic modulation in chronic heart failure and chronic obstructive pulmonary disease. *Arch Med Sci* 2010; **6**: 719-727

- [DOI: 10.5114/aoms.2010.17087]
- 51 **Gupta D**, Nath A, Agarwal R, Behera D. A prospective randomized controlled trial on the efficacy of noninvasive ventilation in severe acute asthma. *Respir Care* 2010; **55**: 536-543 [PMID: 20420722]
  - 52 **Xue Z**, Yu Y, Gao H, Gunst SJ, Tepper RS. Chronic continuous positive airway pressure (CPAP) reduces airway reactivity in vivo in an allergen-induced rabbit model of asthma. *J Appl Physiol* (1985) 2011; **111**: 353-357 [PMID: 21493723 DOI: 10.1152/japplphysiol.01345.2010]
  - 53 **Busk M**, Busk N, Puntenney P, Hutchins J, Yu Z, Gunst SJ, Tepper RS. Use of continuous positive airway pressure reduces airway reactivity in adults with asthma. *Eur Respir J* 2013; **41**: 317-322 [PMID: 22835615 DOI: 10.1183/09031936.00059712]
  - 54 **de Freitas Dantas Gomes EL**, Costa D, Germano SM, Borges PV, Sampaio LM. Effects of CPAP on clinical variables and autonomic modulation in children during an asthma attack. *Respir Physiol Neurobiol* 2013; **188**: 66-70 [PMID: 23681081 DOI: 10.1016/j.resp.2013.05.004]
  - 55 **Mazzone SB**, Canning BJ. Evidence for differential reflex regulation of cholinergic and noncholinergic parasympathetic nerves innervating the airways. *Am J Respir Crit Care Med* 2002; **165**: 1076-1083 [PMID: 11956048 DOI: 10.1164/ajrcm.165.8.2001121270c]

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Case Control Study

# Endoscopic ear surgery: A case series and first United Kingdom experience

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## Abstract

**AIM:** To present the United Kingdom's first case series of 70 otological cases of endoscopic and non-endoscopic ear surgeries.

**METHODS:** Prospective case series incorporating a range of endoscopic procedures performed using a 4 mm, 18 cm rigid endoscope, performed by a single surgeon at a single centre. Primary outcome measures included mean average pre and post-operative air-bone gap hearing thresholds and duration of surgery.

**RESULTS:** Thirty-eight patients underwent endoscopic assisted ear surgery and 32 underwent non-endoscopic assisted ear surgery. In both surgical groups, there was a significant difference between pre and post-operative mean air-bone gaps ( $P = 0.02$ ). Mean operating time was comparable between both groups. Eight patients developed post-operative complications.

**CONCLUSION:** Endoscopic ear surgery can be performed safely in a range of otological procedures. This has the potential to become a well-established surgical option for middle ear surgery in the near future. Advantages and limitations are discussed.

**Key words:** Endoscopic; Mastoid; Surgery; Imaging; Otology; Cholesteatoma

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**Core tip:** The role of endoscopic ear surgery is yet to be properly established but as more otologists adopt this technique, its role will become much more clearly defined and may lead to widespread use based upon positive outcomes for surgery. As with every new surgical technique, a learning curve must first be overcome before reliable conclusions can be drawn about its use. Our series has shown the benefits of using this technique in limited cholesteatoma disease and in providing a good view during revision mastoid surgery with simple pathology.

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## INTRODUCTION

The advantages of using endoscopes in surgery are well described and relate mainly to their portability and ability to provide clear, high quality images<sup>[1]</sup>. Endoscopes can also be used in theatre and the outpatient setting. In particular, the benefits for middle ear surgery include the ability to visualise poorly seen structures, such as the hypotympanum and sinus tympani, which are often an obstacle during the open-technique approach<sup>[2]</sup>. In addition, their use *via* the permeal approach in bypassing a narrow isthmus can provide direct access and a wide view into the middle ear for surgery<sup>[3-5]</sup>. Benefits of using an endoscope can therefore decrease operating time due to the reduction in time need to gain access into the middle ear cleft<sup>[6]</sup> and the subsequent closure at the end of the procedure. The disadvantages of endoscopes used in ear surgery include operator dependence (especially in relation to the one-handed technique), restricted views from narrower endoscopes (e.g., 2.7 mm as compared to 4 mm), the ability to manage complications such as bleeding within a narrower operating field, loss of depth perception, limited magnification, and the need for further training in their use<sup>[4,5]</sup>. Furthermore, when used solely in a permeal approach, the surgeon must use a one-handed technique for instrumentation and there may be difficulty passing other instruments alongside, even in wide ear canals. Certainly there is no scope for using the operating drill in its present form.

Endoscopic ear surgery can be applied to a variety of operations including; grommet insertion, myringoplasty<sup>[2]</sup>, attic retractions<sup>[6]</sup>, cholesteatoma surgery<sup>[7-15]</sup>, stapedectomy<sup>[1,15]</sup>, benign neoplasms of the middle ear<sup>[16]</sup> and neuro-otological procedures<sup>[4,17,18]</sup>. Based on the literature their use has been most commonly described for middle ear disease (cholesteatoma). It has been suggested that preservation of middle ear mucosa by limited surgery using the endoscope can improve the re-aeration of the mastoid cavity leading to better outcomes in surgery<sup>[2]</sup>. There are also roles in "second look" middle ear surgery using 30 degree endoscopes to check for disease clearance<sup>[14,19]</sup>.

Many of the surgeries described above are derived from international case series from France, Germany, Italy, India, UAE, China, Egypt, Iran and the United States<sup>[1,5,12-14,17-24]</sup>. We present the first United Kingdom case series that uses a permeal exclusively endoscopic approach<sup>[20]</sup>.

## MATERIALS AND METHODS

We describe a case series of 70 patients who

underwent either endoscope-assisted or non-endoscope-assisted ear surgery by a single senior surgeon in a district general hospital. Data collection was carried out prospectively for endoscopic cases and retrospectively for non-endoscopic cases where all cases were performed within a 2 year period (2012-2014). A 4 mm diameter, 18 cm long rigid endoscope was used in all cases. Primary outcomes include mean average pre and post-operative air-bone gap hearing thresholds or duration of surgery, depending on the type of surgery. Pre and post-operative audiometric data using both air and bone conduction (at 500 Hz, 1 KHz, 2 KHz and 4 KHz frequencies) was recorded. Complications were noted. Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc, La Jolla, CA, United States).

### Statistical analysis

The statistical methods of this study were reviewed by Virk J, Cambridge University graduate. The dataset was principally descriptive with simple paired t-testing only.

## RESULTS

Seventy patients underwent surgery between the ages of 7-85. Of these, 38 underwent endoscope-assisted ear surgery (Group A) and 32 underwent non-endoscope-assisted ear surgery (Group B). All cases were performed under general anaesthesia. Imaging was reviewed prior to surgery. An endoscope was used exclusively for all patients who underwent endoscope-assisted ear surgery, except in parts of an operation which required the use of a microscope (e.g., mastoid portion of modified radical mastoidectomy or canal wall up mastoidectomy). Procedures in Group B patients were preferentially performed with the microscope such as revision stapedectomies under local anaesthetic or those with extensive disease and the endoscope was not used during the procedure. No cases were converted from endoscopic to open operations. Both groups were matched as closely as possible for type of surgery and demographics.

In Group A, 20 patients had had previous surgery to the operated ear (*i.e.*, ipsilateral ear) compared to 7 patients in Group B. A summary of different operations within Group A and B are shown in Table 1. Tables 2-6 summarise data for each operative group.

Overall, air-bone gap closure was achieved within 10 dB in 9 patients (5 Group A vs 4 Group B), within 10-30 dB in 18 patients (8 Group A vs 10 Group B), over 30 dB in 9 patients (2 Group A vs 7 Group B), over-closure in 5 patients (4 Group A vs 1 Group B) and no change in 25 patients (18 Group A vs 7 Group B). In both groups, there was a significant difference between pre and post-operative mean air-bone gaps ( $P < 0.05$ ) (paired *t* test,  $P = 0.036$  group A and  $P = 0.002$  for group B) for patients who underwent stapedectomy, where air-bone gap was a primary



**Table 1 Summary of procedures**

Procedure (including revision surgery)	Endoscopic assisted	Non-endoscopic assisted
	Group A	Group B
Ventilation Tube insertion	1	2
Myringoplasty, tympanoplasty, ossiculoplasty, Tympanotomy	10	10
CSOM and cholesteotoma surgery	15	10
Stapedectomy	11	9
Petrosectomy	1	1
Total	38	32

**Table 2 Ventilation tube insertion**

No.	Age	Side	Duration (min)	Previous ipsilateral surgery	Pre-op mean air-bone gap <sup>1</sup>	Post-op mean air-bone gap <sup>1</sup>	Closure	Follow up (mo)	Complications
Endoscopic assisted, Group A									
1	15	R	25	No	22.5	0	Within 10-30 dB	4	None
Non-endoscopic assisted, Group B									
1	14	R + L	20	No	25	10	Within 10-30 dB	9	None
2	53	R + L	15	No	20	20	No change	24	Recurrent otitis media with effusion

<sup>1</sup>Mean gap calculated over 4 frequencies (500 Hz, 1 KHz, 2 KHz, 4 KHz).

**Table 3 Myringoplasty, Tympanoplasty, Ossiculoplasty and Tympanotomy**

No.	Age	Details	Side	Duration (min)	Previous ipsilateral surgery	Pre-op mean air-bone gap <sup>1</sup>	Post-op mean air-bone gap <sup>1</sup>	Closure	Graft material	Follow up (mo)	Complications
Endoscopic assisted, Group A											
1	63	Myringoplasty	R	66	Yes	Dead ear	Dead ear	No change	Conchal cartilage	1	Tragal abscess and otitis externa
2	37	Myringoplasty	L	60	No	15.5	12.5	No change	Temporalis fascia	4	None
3	55	Myringoplasty	L	45	Yes	5	5	No change	Composite tragal graft	4	None
4	16	Revision myringoplasty	R	45	Yes	0	0	No change	Tragal cartilage	5	None
5	22	Tympanoplasty	R	88	No	0	0	No change	Composite tragal graft	12	None
6	45	Tympanoplasty	L	101	Yes	20	15	Within 10-30 dB	Tragal cartilage	4	None
7	32	Tympanoplasty	L	98	No	7.5	6.25	Within 10 dB	Tragal cartilage	2	None
8	46	Tympanoplasty	L	111	Yes	18.75	23.3	No change	Tragal cartilage	2	None
9	35	Tympanoplasty	R	121	No	30	11.25	Within 10 dB	Not stated	3	None
10	34	Ossiculoplasty	R	123	Yes	42.5	12.5	Within 10-30 dB	Not stated	10	None
Non-endoscopic assisted, Group B											
1	12	Myringoplasty	R	55	No	12.5	11.25	Within 10-30 dB	5	5	None
2	9	Myringoplasty	L	130	No	27.5	Not available	Not available	Temporalis fascia	Lost to follow up	None
3	30	Revision myringoplasty	L	97	No	16.25	15	Within 10-30 dB	Temporalis fascia	12	None
4	66	Tympanoplasty	L	127	Yes	Not available	Not available	Not available	Not stated	5	Will need ossiculoplasty
5	59	Tympanoplasty	L	114	No	15	40	> 30 dB	Not stated	4	Scarring, false fundus recurrence
6	33	Tympanoplasty	L	174	No	Dead	Dead	No change	Composite tragal graft	5	None
7	10	Tympanoplasty	L	88	No	23.75	21.25	Within 10-30 dB	Temporalis fascia	3	None
8	21	Tympanoplasty	R	92	No	16.25	6.25	Overclosure	Temporalis fascia	10	None
9	63	Tympanoplasty	R	100	No	Dead	Dead	No change	Not stated	3	None
10	50	Revision tympanoplasty	R	101	Yes	0	20	> 30 dB	Tragal cartilage	2	None

<sup>1</sup>Mean gap calculated over 4 frequencies (500 Hz, 1 KHz, 2 KHz, 4 KHz).

outcome.

Mean operating times were as follows; ventilation

tube insertion 25 min vs 17.5 min in (Group A,  $n = 1$  vs Group B,  $n = 2$ ), myringoplasty, tympanoplasty,

**Table 4 CSOM and cholesteatoma surgery**

No.	Age	Details	Side	Duration (min)	Previous ipsilateral surgery	Pre-op mean air-bone gap <sup>1</sup>	Post-op mean air-bone gap <sup>1</sup>	Closure	Graft material	Follow up (mo)	Complications
Endoscopic assisted, Group A											
1	42	Mastoidectomy	L	211	No	7.5	7.5	No change	Temporalis fascia	6	None
2	40	Revision mastoidectomy	R	155	Yes	40	40	No change	Not stated	3	None
3	7	Tympanoplasty and mastoid exploration	L	169	Yes	13.75	5	Overclosure	Conchal cartilage	3	None
4	35	Tympanotomy	L	48	No	25	25	No change	Not stated	2	None
5	18	CWU mastoidectomy	L	195	No	7.5	17.5	Within 10 dB	Conchal cartilage	7	None
6	52	CWU mastoidectomy	L	287	Yes	20	18.75	> 30 dB	Tragal cartilage	6	None
7	13	Revision CWU mastoidectomy	L	188	Yes	11	25	> 30 dB	Tragal cartilage	2	None
8	47	MR mastoidectomy	R	287	No	27.5	23.75	Within 10-30 dB	Not stated	2	None
9	40	MR mastoidectomy	L	223	Yes	Data unavailable	16.25	Within 10-30 dB	Tragal cartilage	2	Post op. pain
10	28	Revision MR mastoidectomy	L	228	Yes	21.25	21.25	No change	Not stated	4	None
11	41	Revision MR mastoidectomy	L	140	Yes	31.25	31.25	No change	Not stated	4	None
12	35	Revision MR mastoidectomy	R	95	Yes	42.5	42.5	No change	Not stated	6	None
13	85	Revision MR mastoidectomy	L	110	Yes	20	20	No change	Not stated	11	None
14	68	Revision MR mastoidectomy	L	155	Yes	45	50	No change	Temporalis fascia	3	None
15	43	Revision MR mastoidectomy		78	Yes	Dead ear	Dead ear	No change	Not stated	4	Transient delayed facial palsy
Non-endoscopic assisted, Group B											
1	8	CWU mastoidectomy	L	220	No	17.5	12.5	Within 10-30 dB	Temporalis fascia	12	None
2	52	CWU mastoidectomy	L	286	No	27.5	25	> 30 dB	Not stated	7	None
3	13	Revision CWU mastoidectomy	L	189	Yes	7.5	30	Within 10-30 dB	Tragal cartilage	5	None
4	70	MR mastoidectomy	L	131	No	13.75	20	> 30 dB	Composite tragal graft	3	None
5	42	MR mastoidectomy	R	255	No	28.75	35	> 30 dB	Temporalis fascia	3	None
6	34	MR mastoidectomy	R	312	No	33.75	37.5	> 30 dB	Composite tragal graft	2	None
7	73	Revision MR mastoidectomy	L	150	Yes	Dead ear	Dead ear	No change	Tragal cartilage	9	None
8	77	Revision MR mastoidectomy	L	179	Yes	2.5	21.25	Within 10-30 dB	Tragal Cartilage	8	None
9	56	Revision MR mastoidectomy	L	251	No	20	10	Within 10-30 dB	Temporalis fascia	4	None
10	78	Revision MR mastoidectomy	R	199	No	Dead ear	Dead ear	No change	Not stated	6	None

<sup>1</sup>Mean gap calculated over 4 frequencies (500 Hz, 1 KHz, 2 KHz, 4 KHz). CWU: Canal wall up mastoidectomy; MR: Modified radical mastoidectomy.

tympanotomy and ossiculoplasty 85.8 min vs and 107.8 min (Group A,  $n = 10$  vs Group B,  $n = 10$ ), CSOM and cholesteatoma surgery 171 min vs 217.2 min (Group A,  $n = 15$  vs Group B,  $n = 10$ ), stapedectomy 136.5 min vs 175.2 min (Group A,  $n = 11$  vs Group B,  $n = 9$ ) and petrosectomy 387 min vs 253 min (Group A,  $n = 1$  vs Group B,  $n = 1$ ).

Graft material was used in a total of 30 patients (15 vs 15 patients from Group A and B respectively). Choice of graft material varied from tragal cartilage (7 vs 5), conchal cartilage (3 vs 0), composite tragal graft (2 vs 4), temporalis fascia (3 vs 6) and fascia lata and fat (1 vs 0) from patients in Group A and B respectively.

**Table 5 Stapedectomy**

No.	Age	Details	Side	Duration (min)	Previous ipsilateral surgery	Pre-op mean air-bone gap <sup>1</sup>	Post-op mean air-bone gap <sup>1</sup>	Closure	Prosthesis	Follow up (mo)	Complications
Endoscopic Assisted, Group A											
1	30	Stapedectomy	L	149	No	28.75	10	Overclosure	SMart piston	9	None
2	57	Stapedectomy	L	119	No	11.25	15	Within 10-30 dB	SMart piston	3	None
3	43	Stapedectomy	R	137	No	35	6.25	Within 10-30 dB	SMart piston	6	None
4	44	Stapedectomy	R	145	No	32.5	10	Within 10-30 dB	SMart piston	4	None
5	32	Stapedectomy	R	150	No	25	26.25	No change	Plastipore PORP	5	None
6	39	Stapedectomy	R	115	No	40	13.75	Within 10 dB	PORP	3	None
7	45	Stapedectomy	R	151	No	38.75	40	Overclosure	Porphexpiston	2	Infection in mastoid cavity
8	33	Stapedectomy	R	125	No	13.75	6.25	Overclosure	SMart piston	5	None
9	37	Revision stapedectomy	L	139	Yes	25	17.5	Within 10 dB	SMart piston	8	None
10	32	Revision stapedectomy	L	142	Yes	60	60	No change	SMart piston	2	Labyrinthitis
11	47	Revision revision stapedectomy	R	129	Yes	16.25	20	Within 10-30 dB	SMart piston	7	None
Non-endoscopic Assisted, Group B											
1	48	Stapedectomy	R	254	No	45	7.5	< 10 dB	fluoroplastic piston	11	None
2	44	Stapedectomy	R	230	No	21.25	Not available	n/a	Fluoroplastic piston	Lost to follow up	
3	45	Stapedectomy	L	118	No	26.25	8.75	< 10 dB	Smart piston	5	None
4	41	Stapedectomy	R	265	No	37.5	13.75	Within 10-30 dB	Smart piston	3	None
5	41	Stapedectomy	L	253	No	33.75	16.25	Within 10-30 dB	Smart piston	13	None
6	42	Stapedectomy	L	98	No	32.5	20	Overclosure	Fluoroplastic piston	22	None
7	40	Stapedectomy	L	169	No	40	5	< 10 dB	Fluoroplastic piston	14	None
8	56	Revision stapedectomy	L	111	Yes	20	10	< 10 dB	Fluoroplastic piston	5	None
9	38	Revision stapedectomy	R	79	Yes	26.25	21.25	> 30 dB	Fluoroplastic piston	8	Planned for revision revision surgery

<sup>1</sup>Mean gap calculated over 4 frequencies (500 Hz, 1 KHz, 2 KHz, 4 KHz). PORP: Partial ossicular replacement prosthesis; n/a: Not Applicable.

Eight patients developed post-operative complications that later resolved including otalgia, recurrent otitis media with effusion, transient delayed facial palsy, labyrinthitis, tragal abscess and tympanic membrane perforation and infection of the mastoid cavity (see Tables 2-6). Three patients were planned for further surgery at follow up. Mean post-operative follow up was 8.8 mo; 2 patients were lost to follow up.

## DISCUSSION

### Ventilation tube insertion

Only one patient in our case series had ventilation tube insertion lasting 25 min. Though numbers are extremely low, and therefore difficult to analyse, this operation took 7.5 min longer than the mean duration of non-endoscope-assisted surgery. In contrast, a recent study examining 260 endoscopic grommet insertions demonstrated operating times between 5 and 10 min in all cases<sup>[2]</sup>. Another study has shown that there is no significant difference in duration compared to using a microscope, though it does

advocate the use of an endoscope when ventilation tube placement is technically difficult<sup>[21]</sup>.

### Myringoplasty, Tympanoplasty and Tympanotomy

This series demonstrates that the endoscope can effectively access the middle ear for these procedures. No further incisions were required and an exclusively permeal approach was used in all endoscopic procedures. Surgical outcomes were good in all cases (Table 2) with shorter mean operating times as compared to Group B (non-endoscope = assisted surgery), 85.8 min vs 107.8 min for Group A vs B respectively. This is a fairly accurate representation of true operating time, since the same numbers of operations were performed in each group. There is also evidence that excellent hearing thresholds can be achieved endoscopically, as reported by Balasubramanian and Venkatesan, who achieved pure tone average hearing thresholds of 20 dB in 50 myringoplasties performed endoscopically, further confirming the efficacy of this technique in selected cases<sup>[2]</sup>.

**Table 6 Petrosectomy**

No.	Age	Side	Duration (min)	Previous ipsilateral surgery	Pre-op mean air-bone gap <sup>1</sup>	Post-op mean air-bone gap <sup>1</sup>	Closure	Graft material	Follow up (mo)	Complications
Endoscopic assisted, Group A										
1	63	R	387	No	Dead ear	Dead ear	No change	Fat, Fascia lata	3	Intraoperative CSF leak; TM perforation
Non-endoscopic assisted, Group B										
1	79	R	253	No	Dead ear	90	No change	Not stated	6	referral for cochlear implant

<sup>1</sup>Mean gap calculated over 4 frequencies (500 Hz, 1 KHz, 2 KHz, 4 KHz). CSF: Cerebrospinal fluid; TM: Tympanic membrane.

### CSOM and cholesteatoma surgery

Mean operating time was shorter in Group A compared to Group B (171 min vs 217.2 min respectively). Since total number of operations here were not equal ( $n = 15$  vs  $n = 10$ ), it is unreliable to claim the difference between these figures is of clinical significance. Variation in anatomy and pre-operative disease state (*e.g.*, actively discharging ear), will also have implications on duration of operation due to technical difficulty.

Cholesteatoma can vary in anatomical spread and severity of disease. In widespread, severe cases, canal wall up mastoidectomy or modified radical mastoidectomy can be performed. Our case series shows a variation in the number of these procedures between both groups. Performing mastoidectomy exclusively with an endoscope is impossible, and therefore drawing comparisons between these groups is difficult, as the endoscope will not have been used during a proportion of surgery in Group A. However, only the endoscope was used for the entire operation where there were cases of limited cholesteatoma, or recurrent disease in revision surgery. Recent literature supports this, demonstrating that an exclusively endoscopic approach can be very useful as a "second look" surgery in in order to identify residual cholesteatoma<sup>[11]</sup>.

The most widely documented use of endoscopic ear surgery has been for cholesteatoma disease. Some studies have examined the use of the endoscope as an adjunct for surgery. Residual cholesteatoma rates in closed cavity surgery have been documented around 9%, which is comparable to use with a microscope alone<sup>[14]</sup>. One study examined its use peri-operatively after surgery using the microscope. Residual disease was identified in 65/80 cases, and was documented to commonly occur on the stapes footplate, the stapes crura, and the sinus tympani<sup>[13]</sup>. The use of the endoscope has also been shown to decrease the rate of "open tympanoplasty" during this surgery. Results for localised attic disease have achieved air bone gap closure within 20 dB in around 90% of patients between 3 and 6 years follow up. Figures of 80% disease-free follow up have also been documented on 27 cases with limited attic retractions<sup>[6]</sup>. Our series demonstrates relatively efficient use of the

endoscope during revision surgery, which highlights the importance of a good visibility during technically challenging operations within the middle ear. However longer follow up is required to confirm its efficacy in these revision cases.

### Stapedectomy

Our case series of 11 stapedectomies performed using a 4 mm endoscope at 0 and 30 degrees demonstrated the preservation of the chorda tympani all cases, as well as achieving significant improvement in pre and post-operative air-bone closure ( $P < 0.05$ ) where thresholds were within  $< 30$  dB for all cases. By comparison, the 9 operations performed without the endoscope, also show significant improvement in pre and post-operative air bone gap ( $P < 0.05$ ), but with a longer mean duration of surgery (136.4 min vs 175.2 min for Group A and B respectively). First described by Poe in 2000, endoscopic stapedectomy has gone on to show promise in other countries across the world, achieving significant improvement in air bone gap by comparing pre and post-operative hearing thresholds<sup>[1,15]</sup>. Our series is in keeping with this.

### Petrosectomy

There are some reports of successful use of the endoscope during cholesteatoma surgery within the petrous apex, as was used for one case in our series<sup>[22]</sup>. Due to the discovery of a CSF leak intra-operatively, the duration of surgery is much higher compared to the non-endoscopic assisted surgery. It is difficult to compare these two surgical approaches for this operation from this single case series.

### Clinical applicability

Endoscopic surgery has also been used in a variety of neuro-otological procedures, including acoustic neuroma surgery. Some centres have also begun using it as the first surgical option or as an adjunct to conventional posterior tympanotomy approach in cochlear implantation<sup>[23-25]</sup>. Its benefit as an adjunct to conventional surgical techniques where wider exposure is required due to a limited direct vision has been well recognised<sup>[4,17,18]</sup>. Cadaveric studies using the endoscope alone have also documented superior views of the internal acoustic meatus over conventional



techniques, although clinical applicability for this may well take several years to develop<sup>[23,25]</sup>.

The endoscopic technique in ear surgery undoubtedly gives better quality images and access to blind sacs around the middle ear space that would otherwise not have been visualised adequately using a microscope, irrespective of surgical approach. It is minimally invasive thus providing better cosmesis in patients who do not wish to have a scar. Its use in the outpatient setting has gained popularity by consultant otolaryngologists and junior trainees due to its accessibility, portability and superiority over hand drawn diagrams of the tympanic membrane, which often can be unreliable and inaccurate. In addition, our series demonstrates a role in revision mastoid surgery in particular, where, for example, the cavity can be revised by curettage of a high "facial ridge" entirely endoscopically and permeatally.

The most commonly used rigid endoscopes are 18 cm long and 4 mm (as used for all operations in this case series). Some surgeons find this endoscope difficult to manoeuvre due to its length and larger diameter, and advocate using a paediatric nasal endoscope which is 2.7 mm diameter and 11 cm long<sup>[1]</sup>. However these endoscopes generate poor views and 3 mm endoscopes are available and better suited for ear surgery. Ideally, an endoscope with a small diameter, and shorter length, possibly with a modification to allow the surgeon to keep two hands free but that retains light intensity within a wider field, would be ideal for operating on the middle ear.

In addition, it is worth noting that there is a learning curve when using any new technique. This may be improved for otolaryngologists where we regularly use the endoscope during endoscopic sinus surgery for example.

### Limitations

The numbers for each operation in our prospective case series is low, leaving the study underpowered. However, this case series serves as a pilot study to open the debate of endoscopic ear surgery in the United Kingdom. To enhance our results, more cases would need to be examined in a similar prospective fashion. Only then could reliable conclusions be drawn from comparing endoscopic and open techniques.

Another limitation is the small number in each group, addressed above in regard of the power of the study, alongside the groups being somewhat heterogeneous particularly in the largest group of mastoid and tympanoplasty surgery. However we need to group the surgeries into a grading from simple to complex and these groupings certainly serve to follow this. The groupings, like the above point, serve to illustrate the possibilities of the endoscope rather than to compare the surgeries themselves. Likewise, including in our series, grommet insertion and petrosectomy demonstrates the utility of the

endoscope, despite the few numbers. This will be of value and interest to the readership to investigate further despite the small numbers.

The role of endoscopic ear surgery is yet to be properly established but as more otologists adopt this technique, its role will become much more clearly defined and may lead to widespread use based upon positive outcomes for surgery. As with every new surgical technique, a learning curve must first be overcome before reliable conclusions can be drawn about its use. Our series has shown the benefits of using this technique in limited cholesteatoma disease and in providing a good view during revision mastoid surgery with simple pathology.

## COMMENTS

### Background

Endoscope assisted ear surgery is increasingly common. However its role has not been properly elucidated. The authors investigate potential roles across a range of otological procedures.

### Research frontiers

Minimal access surgery from robotic to endoscopic approaches are being increasingly analysed.

### Innovations and breakthroughs

This study highlights the role of endoscope surgery in revision mastoid surgery alongside the more well-established role in stapedectomy. The endoscope allows excellent visualisation of the middle ear cleft and any cholesteatoma.

### Applications

The endoscope can assist in mastoid surgery, particularly in revision cases. It also has a role in stapedectomy and other middle ear surgery.

### Peer-review

Authors described their experience about endoscopic ear surgery. As mentioned by authors, this surgical procedure has already been described in case series numerically significant.

## REFERENCES

- 1 **Sarkar S**, Banerjee S, Chakravarty S, Singh R, Sikder B, Bera SP. Endoscopic stapes surgery: our experience in thirty two patients. *Clin Otolaryngol* 2013; **38**: 157-160 [PMID: 23164290 DOI: 10.1111/coa.12051]
- 2 **Balasubramanian T**, Venkatesan U. Endoscopic Otolaryngology A supplement. *Otolaryngology* 2012; **2**: 1-25. Available from: URL: [http://opendepot.org/745/1/Endoscopic\\_otology.pdf](http://opendepot.org/745/1/Endoscopic_otology.pdf)
- 3 **Tarabichi M**. Open Access Atlas of Otolaryngology, Head & Neck Operative Surgery. Cited: 2014-01. Available from: URL: <http://www.entdev.uct.ac.za/guides/open-access-atlas-of-otolaryngology-head-neck-operative-surgery/>
- 4 **Bottrill ID**, Poe DS. Endoscope-assisted ear surgery. *Am J Otol* 1995; **16**: 158-163 [PMID: 8572114]
- 5 **Tarabichi M**. Endoscopic middle ear surgery. *Ann Otol Rhinol Laryngol* 1999; **108**: 39-46 [PMID: 9930539]
- 6 **Marchioni D**, Alicandri-Ciufelli M, Molteni G, Genovese E, Presutti L. Endoscopic tympanoplasty in patients with attic retraction pockets. *Laryngoscope* 2010; **120**: 1847-1855 [PMID: 20623791 DOI: 10.1002/lary.21069]
- 7 **Marchioni D**, Villari D, Alicandri-Ciufelli M, Piccinini A, Presutti L. Endoscopic open technique in patients with middle ear cholesteatoma. *Eur Arch Otorhinolaryngol* 2011; **268**: 1557-1563 [PMID: 21336608 DOI: 10.1007/s00405-011-1533-y]
- 8 **Marchioni D**, Mattioli F, Alicandri-Ciufelli M, Presutti L. Prevalence of ventilation blockages in patients affected by attic pathology: a case-control study. *Laryngoscope* 2013; **123**:

- 2845-2853 [PMID: 24037903 DOI: 10.1002/lary.24165]
- 9 **Tarabichi M.** Endoscopic management of cholesteatoma: long-term results. *Otolaryngol Head Neck Surg* 2000; **122**: 874-881 [PMID: 10828802]
- 10 **Badr-el-Dine M.** Value of ear endoscopy in cholesteatoma surgery. *Otol Neurotol* 2002; **23**: 631-635 [PMID: 12218610]
- 11 **Marchioni D,** Villari D, Mattioli F, Alicandri-Ciufelli M, Piccinini A, Presutti L. Endoscopic management of attic cholesteatoma: a single-institution experience. *Otolaryngol Clin North Am* 2013; **46**: 201-209 [PMID: 23566906 DOI: 10.1016/j.otc.2012.10.1004]
- 12 **Tarabichi M,** Nogueira JF, Marchioni D, Presutti L, Pothier DD, Ayache S. Transcanal endoscopic management of cholesteatoma. *Otolaryngol Clin North Am* 2013; **46**: 107-130 [PMID: 23566900 DOI: 10.1016/j.otc.2012.10.001]
- 13 **Ayache S,** Tramier B, Strunski V. Otoendoscopy in cholesteatoma surgery of the middle ear: what benefits can be expected? *Otol Neurotol* 2008; **29**: 1085-1090 [PMID: 18836388 DOI: 10.1097/MAO.0b013e318188e8d7]
- 14 **Yung MW.** The use of middle ear endoscopy: has residual cholesteatoma been eliminated? *J Laryngol Otol* 2001; **115**: 958-961 [PMID: 11779323]
- 15 **Migirov L,** Wolf M. Endoscopic transcanal stapedotomy: how I do it. *Eur Arch Otorhinolaryngol* 2013; **270**: 1547-1549 [PMID: 23463349 DOI: 10.1007/s00405-013-2420-5]
- 16 **Marchioni D,** Alicandri-Ciufelli M, Gioacchini FM, Bonali M, Presutti L. Transcanal endoscopic treatment of benign middle ear neoplasms. *Eur Arch Otorhinolaryngol* 2013; **270**: 2997-3004 [PMID: 23377229 DOI: 10.1007/s00405-013-2371-x]
- 17 **Rosenberg SI,** Silverstein H, Willcox TO, Gordon MA. Endoscopy in otology and neurotology. *Am J Otol* 1994; **15**: 168-172 [PMID: 8172296]
- 18 **Rosenberg SI.** Endoscopic otologic surgery. *Otolaryngol Clin North Am* 1996; **29**: 291-300 [PMID: 8860927]
- 19 **Sajjadi H.** Endoscopic middle ear and mastoid surgery for cholesteatoma. *Iran J Otorhinolaryngol* 2013; **25**: 63-70 [PMID: 24303422]
- 20 **Badr-El-Dine M,** James AL, Panetti G, Marchioni D, Presutti L, Nogueira JF. Instrumentation and technologies in endoscopic ear surgery. *Otolaryngol Clin North Am* 2013; **46**: 211-225 [PMID: 23566907]
- 21 **Nassif N,** Redaelli De Zinis LO, Berlucchi M, Zanetti D. Endoscopic ventilation tube placement in the pediatric age. *Clin Otolaryngol* 2014; **39**: 50-53 [PMID: 24438199 DOI: 10.1111/coa.12221]
- 22 **Aubry K,** Kania R, Sauvaget E, Huy PT, Herman P. Endoscopic transsphenoidal approach to petrous apex cholesteatoma. *Skull Base* 2010; **20**: 305-308 [PMID: 21311627 DOI: 10.1055/s-0030-1249573]
- 23 **Marchioni D,** Grammatica A, Alicandri-Ciufelli M, Genovese E, Presutti L. Endoscopic cochlear implant procedure. *Eur Arch Otorhinolaryngol* 2014; **271**: 959-966 [PMID: 23595616 DOI: 10.1007/s00405-013-2490-4]
- 24 **Migirov L,** Shapira Y, Wolf M. The feasibility of endoscopic transcanal approach for insertion of various cochlear electrodes: a pilot study. *Eur Arch Otorhinolaryngol* 2014 Mar 12; Epub ahead of print [PMID: 24619204]
- 25 **Marchioni D,** Alicandri-Ciufelli M, Mattioli F, Nogueira JF, Tarabichi M, Villari D, Presutti L. From external to internal auditory canal: surgical anatomy by an exclusive endoscopic approach. *Eur Arch Otorhinolaryngol* 2013; **270**: 1267-1275 [PMID: 23010794 DOI: 10.1007/s00405-012-2137-x]

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## May-Thurner syndrome: High output cardiac failure as a result of iatrogenic iliac fistula

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### Abstract

May-Thurner syndrome (MTS) also termed ilio caval compression or Cockett-Thomas syndrome is a common, although rarely diagnosed, condition in which the patient has an anatomical variant wherein the

right common iliac artery overlies and compresses the left common iliac vein against the fifth lumbar spine resulting in increased risk of iliofemoral deep venous thrombosis. This variant has been shown to be present in over 23% of the population but most go undetected. We present a patient with MTS who developed high output cardiac failure due to an iatrogenic iliac fistula. The patient underwent an extensive workup for a left to right shunt including MRI and arterial duplex in the vascular lab. He was ultimately found to have a 2.1 cm left common iliac artery aneurysm and history of common iliac stent. We took the patient to the operating room for aortogram with placement of an endovascular plug of the left internal iliac artery and aorto-bi-iliac stent graft placement with CO<sub>2</sub> and IV contrast. Subsequently the patient underwent successful stent placement in the area that was compressed followed by 6 mo of anticoagulation with warfarin. The flow from the fistula decreased significantly.

**Key words:** May-Thurner syndrome; Cardiac failure; Echocardiogram; Cockett-Thomas syndrome; Iliocaval compression

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**Core tip:** To our knowledge, we describe the first case of high output cardiac failure due to iatrogenic iliac fistula and its management in the setting of May-Thurner syndrome (MTS). In our case, an iatrogenic iliac fistula resulted because of prior stent placement in left iliac vein to prevent deep venous thrombosis (DVT) secondary to MTS. We favored aorto-bi-iliac stent graft placement to prevent the fistula from leaking. In our case, the prior vascular stent placement was a clue to search for the fistula. It is important to note that stent placement to prevent DVT in MTS may result in iatrogenic fistula formation.

Singh S, Singh S, Jyothimallika J, Lynch TJ. May-Thurner

syndrome: High output cardiac failure as a result of iatrogenic iliac fistula. *World J Clin Cases* 2015; 3(3): 318-321 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v3/i3/318.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v3.i3.318>

## INTRODUCTION

May Thurner syndrome (MTS) or ilio caval compression or Cockett-Thomas syndrome is a fairly common well recognized and rarely diagnosed condition. McMurrich first described this anatomical variant in 1908 and believed the variant was the result of "congenital adhesions" in the common iliac veins. May and Thurner published this syndrome in 1957 and it was widely recognized as MTS in United States. In Europe, however, Cockett, a British vascular surgeon and Thomas published the condition in 1965 and it was termed Cockett-Thomas Syndrome. May and Thurner postulated that the chronic pulsations of the overlying right iliac artery led to development of a "spur" in the vein wall and that this spur would result in partial venous obstruction.

## CASE REPORT

A 61 years old male was admitted to our cardiology service for shortness of breath associated with hemodynamic instability (systolic blood pressure, 95mm Hg and diastolic blood pressure, 59 mmHg). His past medical history is significant for hypertension, congestive heart failure, coronary artery disease (post coronary artery bypass graft - 1999), MTS [treated with left iliac vein stent in absence of PE and deep venous thrombosis (DVT) in 2001], chronic pulmonary embolism, protein C deficiency, restrictive lung disease and mild obstructive lung disease. Additionally, the patient smokes half a pack daily for 30 years.

His immediate physical examination revealed hypotension (105/46 mmHg), jugular venous distension of 6 cm, 2/6 pansystolic murmur at the apex, and bilateral pedal edema was noted. Abdomen was soft and non-tender but was distended with normoactive bowel sounds and and liver edge 1 cm below the costal margin. A chest x ray showed mild cardiomegaly with prominence of the central pulmonary arteries (Figure 1). Initial BNP and fibrinogen levels were both elevated (166.7 hh pg/mL and 553 mg/dL). Other blood tests supported renal insufficiency (creatinine 1.68 mg/dl). Echocardiogram revealed diffuse hypokinesia with a low ejection fraction (35%), cardiac output (11.5 L/min), cardiac index (5.54 L/min per square), end diastolic volume (183 mL), end Systolic volume (101 mL), concentric left ventricular hypertrophy, abnormal diastolic relaxation and mild to moderate mitral regurgitation.

Vascular surgery was consulted for evaluation of a possible pelvic shunt/fistula. After extensive workup for

a left to right shunt including MRI and arterial duplex, the patient was found to have a 2.1 cm left common iliac artery aneurysm (21.4 mm) (Figure 2). An Inferior Vena Cava duplex and computed tomography (CT) of the pelvis found evidence of atriovenous fistula at the left iliac vein (discovered 11 years after the initial stent was placed in 2001) (Figure 3).

The patient was taken for an aortogram and heparinized with 5000 units of IV heparin. The right common femoral artery was then accessed and a 5-French sheath was advanced over the wire. An Omniflush catheter was then positioned at the level of L1. The patient had evidence of a high volume fistula from within the left common and hypogastric artery on CO<sub>2</sub> aortogram. Given this finding, an additional magnified view was obtained with the Omniflush catheter positioned at the aortic bifurcation. The Glidewire and Omniflush catheter were then used to select the left hypogastric artery. A 6-French Balkan sheath was brought up and over Magic Torque wire and into the left hypogastric artery. The Amplatz 16 mm plug was then advanced into the distal hypogastric artery. This appeared to be distal to any evidence of the fistula. The plug was then deployed in proper position. Next, a marker pigtail catheter was advanced from the right groin. The left groin cutdown was then performed, using a transverse incision. A 5-French sheath was advanced over the wire. A J-wire was advanced into the descending thoracic aorta. This was exchanged for a Lunderquist wire over the stiff wire. A CO<sub>2</sub> aortogram was again performed. This demonstrated the level of the renal arteries. The main body device, Cook Zenith TFB-28-74 was brought over the Lunderquist wire in the left groin. This was deployed down to the level of the contralateral gate. The pigtail catheter was then pulled down and the top cap was deployed securing the position of the graft. Next, a Kumpe catheter was used to select the contralateral limb from the right femoral sheath. The pigtail catheter was spun confirming proper position. An Amplatz wire was advanced from the right groin. A retrograde injection of contrast was performed from the right groin. Next, the contralateral limb, which was a Cook ZSLE-20-39-ZT, was then deployed with care taken to preserve flow to the right hypogastric artery. Next, the remainder of the main body device was deployed from the left groin. The top cap was then retrieved, and the sheath was removed. Due to the incompetence of a valve, the sheath was then replaced with a Gore DrySeal 18-French Sheath. Next, a retrograde injection of contrast was performed from the left groin. The ipsilateral limb, which was a Cook ZSLE-13-90-ZT was brought onto the field and prepped. This was deployed with care taken to ensure 5 cm of overlap into the left external iliac artery. A 32-French Coda balloon was then used to balloon the proximal and distal sites of fixation, as well as all zones of overlap. Wires were exchanged for a soft wire. This demonstrated evidence of a persistent flow within





Figure 1 Chest X-ray showing mild cardiomegaly with prominence of central pulmonary arteries.



Figure 2 Magnetic resonance imaging of pelvis mark "A" shows 21.4 mm diameter of left common iliac artery.

the fistula that appeared to be less than previous. A completion aortogram was performed. By the end of the procedure cardiac output and cardiac index returned to normal and patient remained stable. Flow through the fistula stopped.

## DISCUSSION

MTS is a fairly common, well recognized and rarely diagnosed condition<sup>[1]</sup>. McMurrich believed the variant was the result of "congenital adhesions" in the common iliac veins<sup>[2]</sup>. May and Thurner published this syndrome in 1957 and it was widely recognized as MTS in United States whereas Cockett and Thomas published it in 1965 and called the same condition Cockett-Thomas Syndrome in Europe<sup>[3]</sup>. May and Thurner postulated that the chronic pulsations of the overlying right iliac artery led to development of a "spur" in the vein wall and that this spur would result in partial venous obstruction<sup>[4]</sup>. Of 430 cadavers, 22% were diagnosed with spurs on left side which is eight times more common than on the right. This came a century after Virchow (1851) first described that thrombosis on the left side was five times more common than on the right side<sup>[5]</sup>. More recently Kibbe *et al*<sup>[6]</sup> demonstrated *via* CT the incidence of MTS in

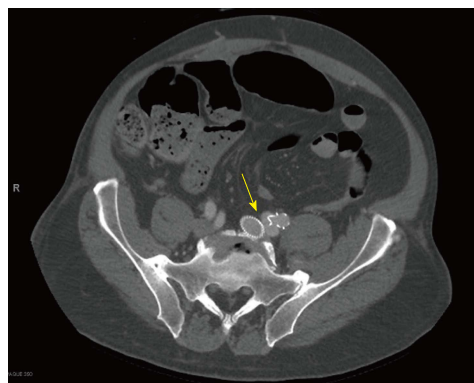


Figure 3 Computed tomography pelvis showing atriovenous fistula of left iliac vessels (yellow arrow) and stent in abdominal aorta.

asymptomatic patients that correlated with autopsy results reported in the early half of twentieth century.

The goal of treatment of MTS is to reduce symptoms and to reduce the risk of complications. The majority of treatments are geared towards treating DVT. The first known report of treatment of MTS solely by endovascular means was by Berger *et al*<sup>[7]</sup> in 1995, who successfully placed a venous stent to relieve iliac compression. The initial step in the treatment of DVT in the setting of MTS is thrombectomy with stent placement<sup>[8]</sup>. Vena Cava filters may be a treatment option for select patients who cannot take anticoagulant medications. Vena Cava filters may not always be used in the treatment of MTS but are used to prevent complications of DVT.

To our knowledge, ours is the first case to report high output cardiac failure due to iatrogenic iliac fistula and its management in MTS. In this case, an iatrogenic iliac fistula resulted because of prior stent placement in left iliac vein to prevent DVT secondary to MTS. We favored aorto-bi-iliac stent graft placement to prevent the fistula from leaking. We managed to successfully reduce cardiac output (11.5 L/min to 8.3 L/min) and cardiac index (5.54 L/min per m sq to 4.0 L/min per sq).

It is important for the practicing physician to note that the identification of high output cardiac failure should lead to a search for the source. In our case, the prior vascular stent placement was a clue to search for the fistula. It is also important to note that stent placement to prevent DVT in MTS can result in iatrogenic fistula.

## ACKNOWLEDGMENTS

We thank Prashant Bhensdadia (MD) (Division of Cardiology, Wake Forest Health, 27157), for bringing to notice this interesting case.

## COMMENTS

### Case characteristics

The patient presented with shortness of breath and bilateral pedal edema.

**Clinical diagnosis**

The patient was found to high output cardiac failure due to iliac fistula resulting from prior management of May-Thurner syndrome.

**Differential diagnosis**

Based on patient history, physical exam, and imaging the authors were able to narrow down on the diagnosis by ruling out severe anemia, paget's disease of bone, hyperthyroidism and beriberi.

**Imaging diagnosis**

The images were obtained by angiography and chest X ray.

**Treatment**

The patient underwent angiography and stent placement.

**Related reports**

Berger A, Jaffe JW, York TN. Iliac compression syndrome treated with stent placement. *J Vasc Surg* 1995; 21: 510-514.

**Experiences and lessons**

It is also important to note that stent placement to prevent deep venous thrombosis in May-Thurner syndrome can result in iatrogenic fistula.

**Peer-review**

This is an interesting case. English well written, understandable and easily readable.

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**REFERENCES**

- 1 Peters M, Syed RK, Katz M, Moscona J, Press C, Nijjar V, Bisharat

M, Baldwin D. May-Thurner syndrome: a not so uncommon cause of a common condition. *Proc (Bayl Univ Med Cent)* 2012; 25: 231-233 [PMID: 22754121]

- 2 McMurrich JP. The occurrence of congenital adhesions in the common iliac veins and their relation to thrombosis of the femoral and iliac veins. *Am J Med Sci* 1908; 135: 342-346 [DOI: 10.1097/0000441-190803000-00004]
- 3 Cockett FB, Thomas ML. The iliac compression syndrome. *Br J Surg* 1965; 52: 816-821 [PMID: 5828716]
- 4 May R, Thurner J. The cause of the predominantly sinistral occurrence of thrombosis of the pelvic veins. *Angiology* 1957; 8: 419-427 [PMID: 13478912]
- 5 Virchow R. Über die Erweiterung kleiner Gefäße. *Arch Path Anat* 1851; 3: 427-462
- 6 Kibbe MR, Ujiki M, Goodwin AL, Eskandari M, Yao J, Matsumura J. Iliac vein compression in an asymptomatic patient population. Presented at the Twenty-seventh Annual Meeting of the Midwestern Vascular Surgical Society; 2003 Sep 18-20; Chicago, Ill, USA
- 7 Berger A, Jaffe JW, York TN. Iliac compression syndrome treated with stent placement. *J Vasc Surg* 1995; 21: 510-514 [PMID: 7877235]
- 8 Mickley V, Schwagierek R, Rilinger N, Görlich J, Sunder-Plassmann L. Left iliac venous thrombosis caused by venous spur: treatment with thrombectomy and stent implantation. *J Vasc Surg* 1998; 28: 492-497 [PMID: 9737459]

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## Intraparotid facial nerve schwannoma: A case report

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**Ethics approval:** This study was case report and so no approval was taken from our institute All India Institute of Medical Sciences, New Delhi.

**Informed consent:** Consent was taken from the patient at the time of carrying out all their investigations, not again at the time of writing case report.

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imaging (MRI) and subsequent surgical excision of the lesion. The lesion showed hyperintensity on T2-weighted and diffusion-weighted MRI. There was no adjacent lymphadenopathy. Although hyperintensity on diffusion-weighted MRI could suggest malignant tumours, the characteristic "string sign" provided the clue for the diagnosis of schwannoma.

**Key words:** Parotid; Facial nerve; Schwannoma; String sign; Imaging

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**Core tip:** There is a difference in an approach to surgery for benign and malignant parotid masses. For benign lesions, superficial parotidectomy is done; whereas in a case of malignant tumour total parotidectomy is performed with or without excision of the facial nerve. Clinically, it is very difficult to differentiate them because even malignant tumours have slow growth. Hence, here comes the role of imaging which could suggest the nature of the mass and narrow the differentials.

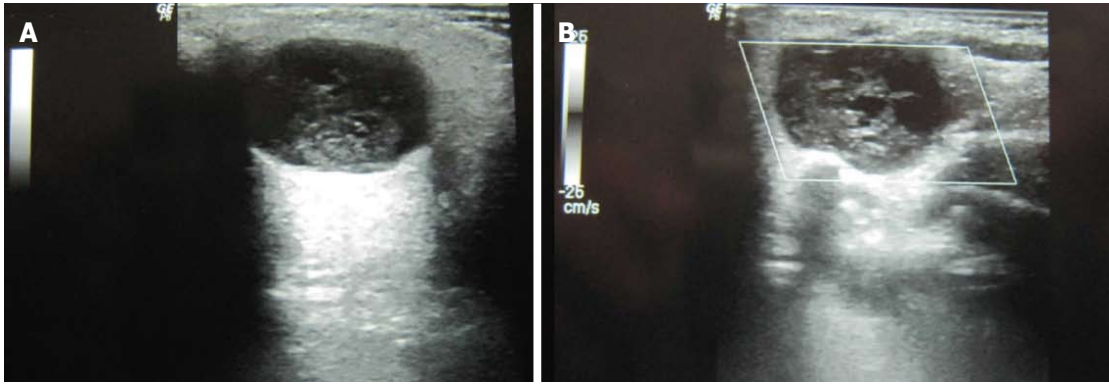
Jaiswal A, Mridha AR, Nath D, Bhalla AS, Thakkar A. Intraparotid facial nerve schwannoma: A case report. *World J Clin Cases* 2015; 3(3): 322-326 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v3/i3/322.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v3.i3.322>

## INTRODUCTION

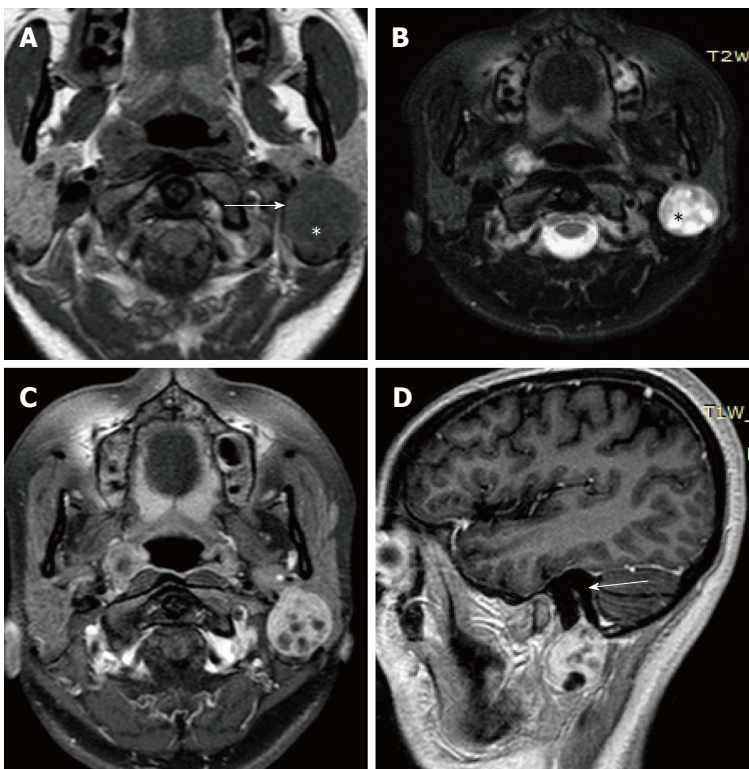
Schwannomas of the Facial nerve (FN) are rare benign encapsulated neurogenic lesions. These can arise anywhere along its course<sup>[1,2]</sup>. Majority of these schwannomas are seen in the intratemporal course of the nerve whereas only 9% are seen in the extratemporal course<sup>[3]</sup>. In a case series of parotid tumours, schwannomas were found to be very rare accounting for only 2 out of 142 lesions<sup>[4]</sup>. As presentation is often nonspecific, preoperative

## Abstract

Facial nerve schwannoma occurring within the parotid gland is a rare tumour. We report a case of schwannoma within the parotid gland in a young female patient, who underwent ultrasound and magnetic resonance



**Figure 1** Ultrasound images. A: Left parotid gland shows presence of well defined hypoechoic mass lesion in the superficial lobe with posterior acoustic enhancement; On Color Doppler (B), no internal vascularity could be demonstrated.



**Figure 2** Magnetic resonance imaging images at the level of parotid glands-axial T1W (A), axial T2W (B), Post Gad T1W images in axial (C) and sagittal (D) planes. Arrow in (A) shows presence of well defined intermediate signal intensity mass lesion in the superficial lobe of left parotid with hypointense areas within it (\*). These regions (\*) are hyperintense on T2W sequence (B) suggestive of myxomatous tissue. On Post contrast images (C and D), the mass enhances homogeneously with few non enhancing areas within. Arrow in D shows the characteristic "string sign" extending along with facial nerve in the stylomastoid foramen.

diagnosis of these tumours is difficult<sup>[4]</sup>.

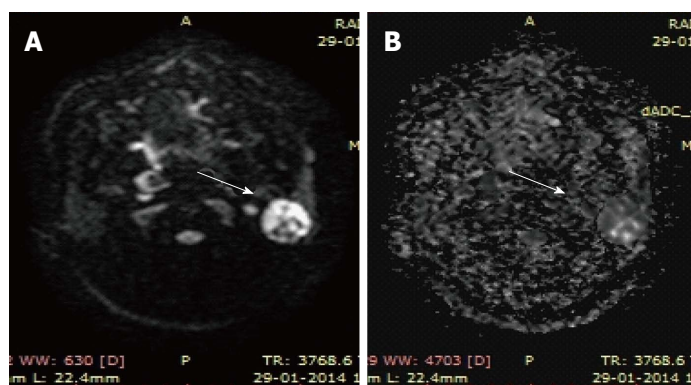
Clinically these patients do not have any facial nerve dysfunction whereas postoperatively features of facial nerve paresis are common. Hence, it becomes extremely necessary for the surgeon to warn the patient regarding this complication beforehand.

## CASE REPORT

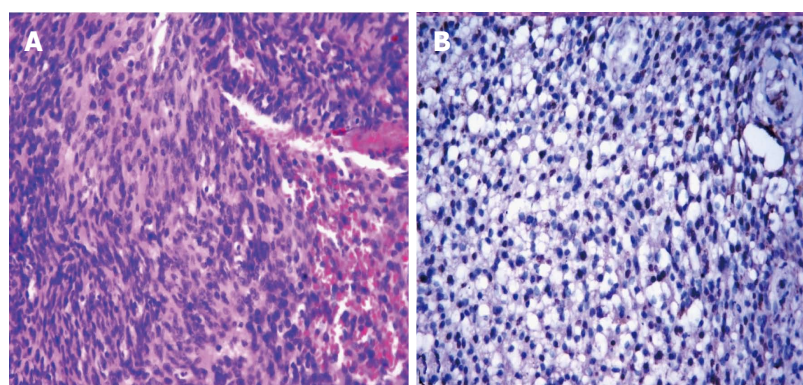
A 27-year-old healthy female presented in the surgical clinic with a slow growing painless swelling in the left retromandibular region for the last one year. There was no history of fever or any other constitutional symptoms. Physical examination revealed a soft, non-tender lump measuring approximately 3 cm × 2 cm. Laboratory tests such as complete haemogram, ESR, CRP were found to be within normal limits.

Ultrasound examination showed the presence of a well defined, hypoechoic mass in the superficial lobe of left parotid which measured approximately 1.8 cm × 2.3 cm (Figure 1A). The mass showed anechoic areas within it with posterior acoustic enhancement suggestive of cystic component. No calcification or adjacent lymphadenopathy was seen. Color Doppler examination (Figure 1B) did not show any internal vascularity. The differential diagnoses were benign pathologies such as pleomorphic adenoma or less likely an intraparotid lymph node. For further characterisation of the lesion, MR examination of the parotid was performed. MR imaging (Figure 2) revealed a well-circumscribed mass lesion in the left parotid gland. The mass was located just below the stylomastoid foramen with a beak like protrusion into it representing the classic "string sign". T1-weighted





**Figure 3** Axial diffusion weighted magnetic resonance imaging. (A) At  $b = 1000 \text{ s/mm}^2$  hyperintensity is noted throughout the mass (arrow) except the cystic areas. These regions were dark (arrow) on ADC map (B) consistent with restricted diffusion.



**Figure 4** Haematoxylin and eosin stained section shows spindle cells arranged in fascicles (A,  $\times 200$ ). The tumour cells are immunoreactive with S-100 protein (B,  $\times 200$ ).

image (Figure 2A) showed the tumour to be of intermediate signal intensity compared to adjacent muscle, and T2-weighted image (Figure 2B) showed high signal intensity with interspersed areas of lower signal intensity. DWI (Figure 3) showed hyperintensity at  $b = 1000 \text{ s/mm}^2$  suggestive of restricted diffusion in the solid part of the lesion with facilitated diffusion in the cystic part. On surgical exploration, the mass was found to be in close relationship with the main trunk of facial nerve just below stylomastoid foramen. Postoperatively, the patient developed mild facial paresis. The tumour was histopathologically confirmed to be schwannoma. The spindle cells were immunopositive with S-100 (Figure 4).

## DISCUSSION

Schwannomas are benign nerve sheath tumours, composed entirely of differentiated neoplastic Schwann cells. Intraparotid FN schwannoma was first reported by Ibarz in 1927. Since then, fewer than 100 cases of FN schwannomas have been reported. In a study by Fortan *et al*<sup>[3]</sup>, majority of the lesions were found within the intratemporal course, whereas about 9% of the tumours were found in the parotid gland<sup>[3]</sup>. The frequency of intraparotid schwannomas range from 0.2% to 1.5%<sup>[5]</sup>. Because of its low prevalence and very few typical clinical and radiological signs associated with it, preoperative diagnosis of intraparotid FN schwannoma is generally difficult.

In a case series of FN schwannomas, the most common clinical manifestation in intratemporal

involvement of the nerve was facial nerve dysfunction, whereas in extratemporal course, it was a parotid mass without facial paresis<sup>[6]</sup>.

In patients with a parotid mass, associated facial nerve palsy generally indicates malignancy. But it can also be seen in benign parotid masses such as pleomorphic adenoma and Warthin's tumour. However, none has been reported in intraparotid schwannoma<sup>[7]</sup>.

Similarly in our case, the patient presented with a parotid mass without facial nerve dysfunction, it thus became very difficult to clinically diagnose the schwannoma without the aid of imaging modalities. Ultrasound evaluation in our case showed a well-defined mass with cystic areas within it. Ultrasound when coupled with newer techniques like elastography can help in differentiating benign from malignant parotid masses<sup>[8]</sup>.

MRI images showed that the mass was situated just below the stylomastoid foramen with beaking into the foramen producing the characteristic "string sign". The string sign is due to the vertical orientation of soft tissue on either ends of the mass. The string represents the normal entering or exiting nerve that is in continuity with the nerve sheath tumour.

MRI features described in four cases of facial nerve schwannomas showed heterogeneous lesions that were isointense to brain on both T1- and T2-weighted images<sup>[9]</sup>. In the present case, the tumour was well defined, isointense and heterogeneously hyperintense to muscle on T1 and T2 weighted images respectively.

Schwannomas may exhibit "target" sign which is characterized by hyperintensity in the periphery

while hypointensity in the centre on T2-weighted images. "Target sign" of neurofibroma is almost pathognomonic<sup>[10]</sup>. This feature is suggestive of neurogenic neoplasm<sup>[11]</sup>. In schwannomas, the target sign is due to compactly packed cellular Antoni A regions which is located centrally and loose myxomatous Antoni B regions in the peripheral part<sup>[11]</sup>. In our case, classical target sign was not observed.

Diffusion weighted imaging features of parotid schwannoma have not been previously described. Restricted diffusion in our case reflects high cellularity of the tumour, supporting the observation that restricted diffusion can be seen in both malignant and benign lesions<sup>[12]</sup>.

Pleomorphic adenomas are the most common tumours of the parotid gland, and a close differential of intraparotid schwannoma due to it being well circumscribed, heterogeneous and hyperintense on T2W sequences<sup>[13]</sup>. But the presence of "string sign" reasonably excluded the possibility of pleomorphic adenoma in our case.

Adenoid cystic carcinoma, another close differential, is a malignant tumour that has the potential to spread along the nerve sheath<sup>[14]</sup>. Malignant tumours are hypointense on T2-weighted images and show ill-defined margins on post contrast images<sup>[15]</sup>. However, T2 hyperintensity and smooth enlargement of the facial nerve canal excludes this diagnosis<sup>[14]</sup>.

In cases of painless swellings of the parotid gland without any neurological involvement, possibility of intraparotid schwannoma should be considered under differentials and the imaging modalities especially MRI revealing characteristic "string sign" further confirms the diagnosis.

## COMMENTS

### Case characteristics

The patient presented with a slow growing painless swelling in the retromandibular region on left side for the last 1 year.

### Clinical diagnosis

The patient's symptoms were nonspecific and presence of painless progressive swelling over a period of 1 year pointed to its benign nature.

### Differential diagnosis

Pleomorphic adenoma was ruled out as there was "string sign" showing extension along the facial nerve into the stylomastoid foramen. Adenoid cystic carcinoma was ruled out as the mass showed T2 hyperintensity and well defined margins. Malignant tumours are T2 hypointense with ill defined margins. Even extension into stylomastoid foramen was accompanied by smooth enlargement of the foramen without any irregular erosion.

### Laboratory diagnosis

Blood tests were non contributory.

### Pathological diagnosis

The excised tumour measured 2.5 cm × 2 cm. Cut surface was fleshy with focal haemorrhage. Microscopic examination showed cellular spindle cells arranged in fascicles. Tumour cells exhibited oval to elongated hyperchromatic nuclei, inconspicuous nucleolus, and fibrillary eosinophilic cytoplasm. Few thick walled blood vessels were seen. No mitosis or necrosis was seen. The tumour cells were immunopositive with S-100; while negative for smooth muscle actin and estrogen receptor. MIB-1 labelling index was < 2%. A diagnosis of schwannoma was given.

## Treatment

Under general anaesthesia, excision of the tumour mass was done and sent for histopathological examination.

## Related reports

Chung SY *et al* article Facial nerve schwannomas: Computed tomography and magnetic resonance findings published in 1998 in *Yonsei Med J* provide a brief but cumulative overview on the case topic.

## Term explanation

Facial nerve schwannoma is a rare neurogenic tumour that arises from the schwann cells of the neurons.

## Experiences and lessons

One lesson that the authors learnt from this case was to consider facial nerve schwannoma in the differential diagnosis of parotid mass when a patient presents with painless progressive swelling and imaging shows characteristic "string sign". Restricted diffusion reflects its high cellularity, supporting the observation that restricted diffusion can be seen in both malignant and benign lesions.

## Peer-review

Good paper.

## REFERENCES

- 1 **O'Donoghue GM**, Brackmann DE, House JW, Jackler RK. Neuromas of the facial nerve. *Am J Otol* 1989; **10**: 49-54 [PMID: 2719087]
- 2 **Symon L**, Cheesman AD, Kawauchi M, Bordi L. Neuromas of the facial nerve: a report of 12 cases. *Br J Neurosurg* 1993; **7**: 13-22 [PMID: 8435140 DOI: 10.3109/02688699308995051]
- 3 **Forton GE**, Moeneclaey LL, Officiers FE. Facial nerve neuroma. Report of two cases including histological and radiological imaging studies. *Eur Arch Otorhinolaryngol* 1994; **251**: 17-22 [PMID: 8179862 DOI: 10.1007/BF00175952]
- 4 **Balle VH**, Greisen O. Neurilemmomas of the facial nerve presenting as parotid tumors. *Ann Otol Rhinol Laryngol* 1984; **93**: 70-72 [PMID: 6703600 DOI: 10.1177/000348948409300116]
- 5 **Chiang CW**, Chang YL, Lou PJ. Multicentricity of intraparotid facial nerve schwannomas. *Ann Otol Rhinol Laryngol* 2001; **110**: 871-874 [PMID: 11558765 DOI: 10.1177/000348940111000912]
- 6 **Chung SY**, Kim DI, Lee BH, Yoon PH, Jeon P, Chung TS. Facial nerve schwannomas: CT and MR findings. *Yonsei Med J* 1998; **39**: 148-153 [PMID: 9587255 DOI: 10.3349/ymj.1998.39.2.148]
- 7 **Nader ME**, Bell D, Sturgis EM, Ginsberg LE, Gidley PW. Facial Nerve Paralysis due to a Pleomorphic Adenoma with the Imaging Characteristics of a Facial Nerve Schwannoma. *J Neurol Surg Rep* 2014; **75**: e84-e88 [PMID: 25083397 DOI: 10.1055/s-0034-1368149]
- 8 **Klintonworth N**, Mantsopoulos K, Zenk J, Psychogios G, Iro H, Bozzato A. Sonoelastography of parotid gland tumours: initial experience and identification of characteristic patterns. *Eur Radiol* 2012; **22**: 947-956 [DOI: 10.1007/s00330-011-2344-7]
- 9 **Martin N**, Sterkers O, Mompont D, Nahum H. Facial nerve neuromas: MR imaging: Report of four cases. *Neuroradiology* 1992; **34**: 62-67 [PMID: 1553040 DOI: 10.1007/BF00588435]
- 10 **Suh JS**, Abenzoza P, Galloway HR, Everson LI, Griffiths HJ. Peripheral (extracranial) nerve tumors: correlation of MR imaging and histologic findings. *Radiology* 1992; **183**: 341-346 [PMID: 1561333 DOI: 10.1148/radiology.183.2.1561333]
- 11 **Murphey MD**, Smith WS, Smith SE, Kransdorf MJ, Temple HT. From the archives of the AFIP. Imaging of musculoskeletal neurogenic tumors: radiologic-pathologic correlation. *Radiographics* 1999; **19**: 1253-1280 [PMID: 10489179 DOI: 10.1148/radiographics.19.5.g99se101253]
- 12 **Qayyum A**. Diffusion-weighted imaging in the abdomen and pelvis: concepts and applications. *Radiographics* 2009; **29**: 1797-1810 [PMID: 19959522 DOI: 10.1148/rg.296095521]
- 13 **Ikeda K**, Katoh T, Ha-Kawa SK, Iwai H, Yamashita T, Tanaka Y. The usefulness of MR in establishing the diagnosis of parotid pleomorphic adenoma. *AJNR Am J Neuroradiol* 1996; **17**: 555-559 [PMID: 8881252]

- 14 **Teresi LM**, Lufkin RB, Wortham DG, Abemayor E, Hanafey WN. Parotid masses: MR imaging. *Radiology* 1987; **163**: 405-409 [PMID: 3562818 DOI: 10.1148/radiology.163.2.3562818]
- 15 **Christe A**, Waldherr C, Hallett R, Zbaeren P, Thoeny H. MR

imaging of parotid tumors: typical lesion characteristics in MR imaging improve discrimination between benign and malignant disease. *AJNR Am J Neuroradiol* 2011; **32**: 1202-1207 [PMID: 21724574 DOI: 10.3174/ajnr.A2520]

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## Rare case of upper gastrointestinal bleeding in achalasia

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**Author contributions:** Xie XJ and Zhan SH supervised the patient's diagnosis and treatment; Zhang WW wrote the manuscript; Geng CX revised the manuscript.

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vomiting blood for six hours. Physical examination revealed that the patient had severe anemia and mild palpitation in the upper abdomen. CT revealed lower esophageal dilatation and esophageal wall thickening, and an emergency upper endoscopy showed that the esophagus was substantially expanded by a dark round stone, with multiple ulcers on the esophageal wall and a slit in the cardiac mucosa with a large clot attached. The patient's history included ingestion of 1 kg hawthorn three days prior. The acute upper gastrointestinal bleeding was caused by Mallory-Weiss syndrome associated with achalasia and an esophageal stone. For patients with achalasia, preventing excessive ingestion of tannins is crucial to avoid complications such as bleeding and rupture.

**Key words:** Achalasia; Esophageal stone; Mallory-Weiss syndrome; Upper gastrointestinal bleeding

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**Core tip:** Achalasia is the prototypic esophageal motility disorder that leaves patients at risk for various complications. This is a rare report of long-term achalasia associated with esophageal stone and ulcer formation leading to upper gastrointestinal bleeding caused by Mallory-Weiss syndrome. This paper highlights the importance of avoiding excess tannin ingestion for patients with achalasia to prevent the development of complications such as bleeding and rupture.

### Abstract

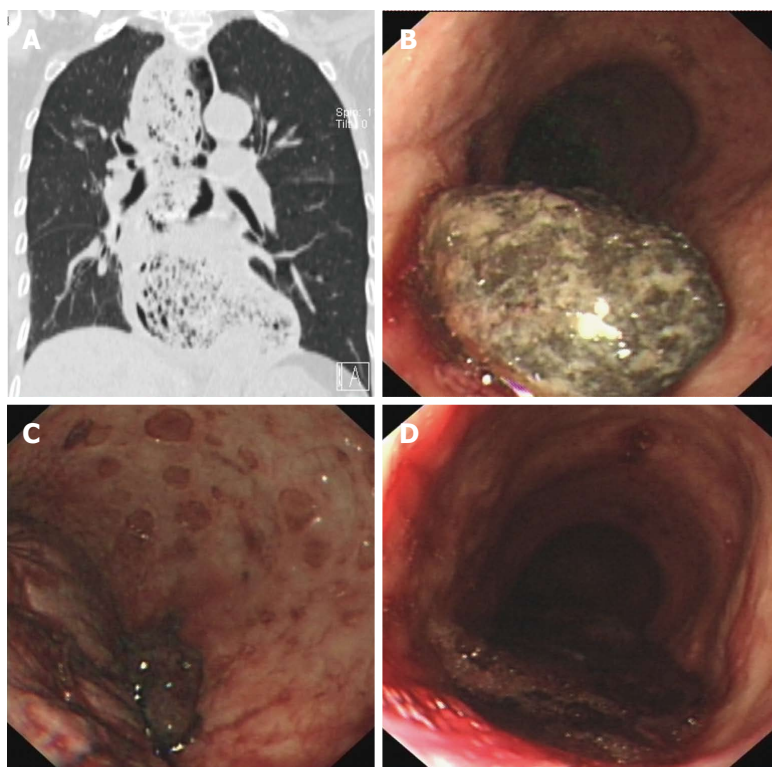
Achalasia is a prototypic esophageal motility disorder with complications including aspiration-pneumonia, esophagitis, esophageal-tracheal fistula, spontaneous rupture of the esophagus, and squamous cell carcinoma. However, achalasia is rarely associated with esophageal stones and ulcer formation that lead to upper gastrointestinal bleeding. Here, we report the case of a 61-year-old woman who was admitted to our department after

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### INTRODUCTION

Achalasia is the prototypic esophageal motility disorder





**Figure 1** CT and endoscopy imaging. A: CT showed lower esophageal dilatation and esophageal wall thickening; Upper endoscopy revealed; B: A large, dark round stone; C: Multiple ulcers on the esophageal wall; D: A slit in the cardiac mucosa with a large clot attached.

characterized by a hypertensive lower esophageal sphincter with incomplete relaxation upon swallowing, accompanied by aperistalsis of the esophageal body<sup>[1]</sup>. Patients with achalasia are at risk for developing complications such as aspiration-pneumonia, esophagitis, esophageal ulcers and bleeding, esophageal-tracheal fistula, spontaneous rupture of the esophagus, and squamous cell carcinoma<sup>[2]</sup>. The prevalence is 10 per 100000 in the United States, involving an equal distribution of men and women of all ages and from all ethnicities<sup>[3]</sup>. We report a rare case of upper gastrointestinal bleeding in a patient with achalasia that was associated with an esophageal stone, ulcer formation and Mallory-Weiss syndrome.

## CASE REPORT

A 61-year-old woman who complained of vomiting blood for six hours was admitted to our department. She experienced dizziness, palpitations, sweating, and fatigue, but did not present with fever, rash or jaundice. Her medical history revealed achalasia that had been present for 30 years, for which she had declined any treatment. When questioned, she reported ingesting 1 kg hawthorn within the past 3 d.

A physical examination indicated that the patient had severe anemia due to pale conjunctiva and nail beds, and mild palpitation in the upper abdomen. Routine blood tests showed: red blood cell count,  $1.34 \times 10^{12}/L$ ; hemoglobin, 41 g/L; white blood cell count,  $11.25 \times 10^9/L$ ; platelet count,  $150 \times 10^9/L$ ; blood urea nitrogen, 15.99 mmol/L; and creatinine, 98  $\mu\text{mol}/L$ . Lower esophageal dilatation and esophageal wall thickening

were revealed upon CT examination (Figure 1A). An emergency upper endoscopy was performed revealing substantial expansion of the esophagus by a dark round stone (Figure 1B), multiple ulcers on the esophageal wall (Figure 1C), and a slit in the cardiac mucosa with a large clot attached (Figure 1D). Endoscopic sprinkling hemostasis and injection of 2.5% sodium bicarbonate were applied to cease the bleeding and dissolve the stone.

## DISCUSSION

In achalasia, injury to the lower esophageal sphincter neurons and the loss of the main functional inhibitory neurotransmitter result in a hypertensive sphincter that loses its ability to relax, leading to stenosis of cardia and lower esophagus expansion. Occasionally, patients will present with persistent food retention, and esophageal stone formation can occur in those who have ingested foods rich in tannins, such as hawthorn and persimmon. The pressure of stones can cause multiple ulcers and even upper gastrointestinal bleeding. The discomfort when swallowing and frequent nausea and vomiting can then lead to the development of Mallory-Weiss syndrome.

Currently, there are no curative treatments for achalasia cardia, rather palliative measures are provided, such as oral nitrates or calcium channel blockers, endoscopic pneumatic dilation, injection of sclerosant substances, or surgery<sup>[4-6]</sup>. However, complications such as bleeding and rupture can be prevented by avoiding excessive ingestion of tannins.

## COMMENTS

**Case characteristics**

A 61-year-old female patient complained of vomiting blood for six hours.

**Clinical diagnosis**

Acute upper gastrointestinal bleeding from Mallory-Weiss syndrome associated with achalasia.

**Laboratory diagnosis**

Red blood cell count,  $1.34 \times 10^{12}/L$ ; hemoglobin, 41 g/L; white blood cell count,  $11.25 \times 10^9/L$ ; platelet count,  $150 \times 10^9/L$ ; blood urea nitrogen, 15.99 mmol/L; creatinine 98  $\mu\text{mol/L}$ .

**Imaging diagnosis**

CT showed lower esophageal dilatation and esophageal wall thickening. Upper endoscopy revealed a large round stone causing substantial expansion of the esophagus and multiple ulcers on the esophageal wall. A slit in the cardiac mucosa was observed with a large clot attached.

**Treatment**

Endoscopic sprinkling hemostasis and injection of 2.5% sodium bicarbonate were applied to cease the bleeding and dissolve the stone.

**Related reports**

Although there are some reports of achalasia combined with esophageal intramural hematoma or esophageal varices, reports of achalasia combined with esophageal stones, ulcer formation and Mallory-Weiss syndrome are rare.

**Term explanation**

Achalasia is characterized by esophageal aperistalsis and impaired relaxation of the lower esophageal sphincter.

**Experiences and lessons**

This report not only presents a rare case of upper gastrointestinal bleeding associated with achalasia, but also aims to inform patients with achalasia to avoid excessive ingestion of tannin-rich foods to prevent related complications.

**Peer-review**

This is a very interesting manuscript reporting a rare case of upper gastrointestinal bleeding as a result of long-term achalasia associated with esophageal stones.

## REFERENCES

- 1 Rohof WO, Hirsch DP, Kessing BF, Boeckxstaens GE. Efficacy of treatment for patients with achalasia depends on the distensibility of the esophagogastric junction. *Gastroenterology* 2012; **143**: 328-335 [PMID: 22562023 DOI: 10.1053/j.gastro.2012.04.048]
- 2 O'Neill OM, Johnston BT, Coleman HG. Achalasia: a review of clinical diagnosis, epidemiology, treatment and outcomes. *World J Gastroenterol* 2013; **19**: 5806-5812 [PMID: 24124325 DOI: 10.3748/wjg.v19.i35.5806]
- 3 Fei L, Rossetti G, Moccia F, Cimmino M, Guerriero L, Romano G, Pascotto B, Orlando F. Definition, incidence and etiology: what's new in the 21st century? *Ann Ital Chir* 2013; **84**: 489-494 [PMID: 24141199]
- 4 Moret  M, Ojembarrena E, Barturen A, Casado I. Treatment of achalasia by injection of sclerosant substances: a long-term report. *Dig Dis Sci* 2013; **58**: 788-796 [PMID: 23179151 DOI: 10.1007/s10620-012-2476-x]
- 5 Meireles OR, Horgan S, Jacobsen GR, Katagiri T, Mathew A, Sedrak M, Sandler BJ, Dotai T, Savides TJ, Majid SF, Nijhawani S, Talamini MA. Transesophageal endoscopic myotomy (TEEM) for the treatment of achalasia: the United States human experience. *Surg Endosc* 2013; **27**: 1803-1809 [PMID: 23525881 DOI: 10.1007/s00464-012-2666-9]
- 6 Bhayani NH, Kurian AA, Dunst CM, Sharata AM, Rieder E, Swanson LL. A comparative study on comprehensive, objective outcomes of laparoscopic Heller myotomy with per-oral endoscopic myotomy (POEM) for achalasia. *Ann Surg* 2014; **259**: 1098-1103 [PMID: 24169175 DOI: 10.1097/SLA.0000000000000268]

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