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REVIEW

Epidemiological link between obesity, type 2 diabetes mellitus and cancer

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Abstract

There exists a complex interaction between obesity, type 2 diabetes mellitus (T2DM) and cancer, and an increase in the incidence of cancer is expected with the growing obesity-diabetes pandemic. The association of cancer with diabetes mellitus and obesity appears to be site-specific, the highest risk being for post-menopausal breast cancer, endometrial cancer, and colorectal cancer. Moreover, there is worsening of hyperglycaemia with the onset of cancer, evidencing a bi-directional link between cancer and diabetes mellitus and the need for monitoring for diabetes in cancer survivors. In this review, we look at the epidemiological evidence from observational studies and Mendelian randomization studies linking obesity, diabetes, and cancer, as well as the complex pathophysiological mechanisms involved, including insulin resistance with associated hyperinsulinaemia, the effect of chronic low-grade inflammation, and the effect of various adipokines that are associated with obesity and T2DM. Additionally, we describe the novel therapeutic strategies, based on their role on the discrete pathophysiological mechanisms involved in the tumourigenesis.

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Core Tip: Cancer is the second most common cause of death globally, and the complex pathogenic mechanisms in the development of cancer are not yet fully elucidated. The interplay between obesity, type 2 diabetes and some forms of cancer are well known for the past few years. With a steady increase in the obesity and diabetes pandemics, the incidence of cancer is expected to increase exponentially in the coming years. This review discusses the complex pathophysiological mechanisms linking these three major disease entities, to enhance clinician awareness across the globe, and proposes emerging potential therapeutic strategies.

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INTRODUCTION

Cancer is the second most common cause of mortality from non-communicable diseases in the world, accounting for nearly 9.6 million deaths in the year 2017[1]. Apart from the excess mortality, cancer also contributed to 233.5 million disability-adjusted life-years in 2017. The incidence of cancer has been rising, due to a rise in the associated risk factors like aging population, obesity, diabetes mellitus, and lifestyle-related factors. Globally, the incidence of obesity has reached pandemic proportions, irrespective of the socioeconomic status and the age group[2]. Rising proportion of individuals with obesity has been the driving force for the diabetes pandemic[3], the incidence/prevalence of which is increasing at a faster rate in low-income and middle-income countries than in high-income countries[4].

There is plenty of evidence supporting the association between cancer and either obesity or diabetes mellitus on an individual basis. A recent study evaluated the impact of combined obesity and diabetes mellitus on cancer risk, by calculating the population attributable fraction (PAF) of incident cancers attributable to obesity and diabetes mellitus[5]. They observed that 5.7% of all incident cancers in 2012 were related to the combined effects of diabetes mellitus and obesity. When they limited their calculation to include only twelve adiposity-related cancers (colorectal cancer, postmenopausal breast cancer, endometrial cancer, gallbladder cancer, pancreatic cancer, liver cancer, kidney cancer, ovarian cancer, gastric-cardia cancer, thyroid cancer, multiple myeloma, and oesophageal adenocarcinoma), and six diabetes-related cancers (colorectal cancer, endometrial cancer, breast cancer, gallbladder cancer, pancreatic cancer, and liver cancer), they observed that 13.5%-15.3% of the cancers were attributable to the combined effects of diabetes mellitus and obesity. The study also observed that nearly one-fourth of the diabetes-related cancers and one-third of the adiposity-related cancers, happened due to a rise in prevalence of these risk factors[5].

As the global burden of obesity and diabetes mellitus is going to rise further, the burden of cancer will continue to increase. Therefore, interventions should be done at multiple levels including individual, community, health-care system, and policy making to prevent the development of cancer from these non-communicable diseases. This review will discuss the epidemiological studies linking obesity and type 2 diabetes mellitus (T2DM) to cancer and will explore the potential pathophysiological mechanisms linking obesity, and T2DM to cancer.

EPIDEMIOLOGICAL STUDIES LINKING OBESITY TO CANCER

The obese population shows an increase in relative risk (RR) for developing various cancers, compared to the non-obese population. A recently published systematic review[6] using the data collected from a meta-analysis of epidemiological studies observed that the RR was highest for endometrial cancer (2.54; 95%CI: 2.11-3.06)[7], followed by renal cancer (1.77; 95%CI: 1.68-1.87)[8]. This was followed by pancreatic cancer (1.48; 95%CI: 1.15-1.92)[9], breast cancer (1.42; 95%CI: 1.30-1.45)[10], liver cancer (1.35; 95%CI: 1.24-1.47)[11], colorectal cancer (1.32; 95%CI: 1.18-1.48)[12], melanoma (1.31; 95%CI: 1.19-1.44)[13], ovarian cancer (1.30; 95%CI: 1.10-1.50)[14], thyroid cancer (1.29; 95%CI: 1.18-1.41)[15], leukaemia (1.26; 95%CI: 1.17-1.37)[13], prostate cancer (1.16; 95%CI: 1.08-1.24)[16], gastric cancer (1.13; 95%CI: 1.03-1.24)[17], and bladder cancer (1.10; 95%CI: 1.06-1.42)[18]. However, a previous study also noted that the obese population has a low RR of getting lung cancer (0.79; 95%CI: 0.73-0.85) compared to the non-obese population, indicating an inverse association[19]. The RR for squamous cell carcinoma, adenocarcinoma, and small cell carcinoma of the lung were 0.68 (95%CI: 0.58-0.80), 0.79 (95%CI: 0.65-0.96), and 0.99 (95%CI: 0.66-1.48) respectively indicating that obesity is protective against all types of lung cancer among both current and former smokers.

Obesity is associated with an increased risk of some cancers and decreased risk of other cancers, suggesting that the association between obesity and cancer clearly depends on the site of the cancer (site-specific association). This suggests that if the epidemiological studies analysing the relationship between obesity and cancer are not adequately stratified for the site of cancer, the associations with less common cancers can be masked. Nearly 4% of all new cancers can be attributed to overweight and obesity (adiposity-related cancers), in which endometrial, postmenopausal breast, and colorectal cancers account for more than 60%[20]. Worldwide, the population attributable fraction (PAF) of cancer related to high body mass index (BMI) was greater among women compared to men (5.4% *vs* 1.9%). Moreover, the countries with very high and high human development index (HDI) had higher PAF (5.3% and 4.8%, respectively), compared to countries with moderate and low HDI (1.6% and 1.0%, respectively)[20]. With increasing rates of obesity at younger age, the adiposity-related cancers are detected at a much younger age.

A dose-response meta-analysis of prospective observational studies reported that each 5 kg weight gain is associated with an increase in the RR for postmenopausal endometrial cancer by 39% among hormone replacement therapy (HRT) non-users (RR 1.39; 95%CI: 1.29-1.49) and by 9% among HRT users (RR 1.09; 95%CI: 1.02-1.16)[21]. Similar weight gain is associated with an increase in RR for postmenopausal ovarian cancer by 13% among HRT non-users (RR 1.13; 95%CI: 1.03-1.23), postmenopausal breast cancer by 11% among HRT non-users (RR 1.11; 95%CI: 1.08-1.13), and colorectal cancer by 9% in men (RR 1.09; 95%CI: 1.04-1.13). Weight gain is also associated with a 42% increase in the RR for renal cancer when the highest and lowest level of adult weight gain are compared (RR 1.42; 95%CI: 1.11-1.81)[21]. However, weight gain is not associated with a rise in colorectal cancer in women, premenopausal breast cancer, postmenopausal breast cancer among HRT users, prostate cancer, and thyroid cancer.

A meta-analysis of one hundred and twenty-six observational cohort studies among breast cancer patients reported that each 5 kg of adult weight gain is associated with a 7% increase in postmenopausal breast cancer (RR 1.07; 95%CI: 1.05-1.09), and each 5 kg/m^2 of gain in BMI is associated with a 17% increase in postmenopausal breast cancer (RR 1.17; 95%CI: 1.11-1.23)[22]. Moreover, each 10 cm increase in waist circumference (WC) and hip circumference (HC), are associated with 11% (RR 1.11; 95%CI: 1.08-1.14) and 6% (RR 1.06; 95%CI: 1.04-1.09) increase in postmenopausal breast cancer, respectively. Furthermore, each 0.1 unit increase in waist-hip ratio is associated with a 10% increase in postmenopausal breast cancer (RR 1.10; 95%CI: 1.05-1.16). The increased risk was noted among hormone receptor positive breast cancers compared to receptor negative breast cancers, and among HRT non-users compared to HRT users. Adult weight gain and BMI gain are not consistently associated with premenopausal breast cancer. Each 5 kg of adult weight loss is associated with a 4% decrease in postmenopausal breast cancer (RR 0.96; 95%CI: 0.88-1.04). The study reported that BMI gain in early adult life (between 18-30 years) is inversely associated with postmenopausal (RR 0.81; 95%CI: 0.75-0.87), and premenopausal (RR 0.86; 95%CI: 0.78-0.96) breast cancer[22].

Another meta-analysis of seven prospective observational studies comprising of 18668 men and 24751 women with a mean age of 62 and 63 years (respectively), with a median follow-up period of 12 years reported 1656 first-incident adiposity-related

cancers including postmenopausal breast, colorectum, lower oesophagus, gastric, liver, gallbladder, pancreas, endometrium, ovary, and kidney cancers[23]. The hazard ratios (HR) for first-incident cancers, *per* standard deviation increment in various adiposity indicators including BMI, WC, HC, and waist-hip ratio (WHR) were calculated. The results were 1.11 (95%CI: 1.02-1.21) for BMI, 1.13 (95%CI: 1.04-1.23) for WC, 1.09 (95%CI: 0.98-1.21) for HC, and 1.15 (95%CI: 1.00-1.32) for WHR. For example, the HR for colorectal cancer for each standard deviation increment in BMI, WC, HC, and WHR are 16%, 21%, 15%, and 20%, respectively. These values are not surprising as WC and WHR are better surrogate markers of visceral fat, than BMI. Moreover, HRT non-users have 20% increased risk *per* standard deviation of BMI, WC, and HC for getting postmenopausal breast cancer, compared to HRT users[23].

A recent prospective study evaluated the effect of weight gain during adult years with or without metabolic dysfunction on the risk of getting adiposity-related cancers[24]. The study reported that, compared to people maintaining a stable weight, those with weight gain of greater than 0.45 kg or 1 pound/year was associated with 38% increase in overall cancer risk (HR 1.38; 95%CI: 1.09-1.76), with women (HR 1.39; 95%CI: 1.03-1.87) having higher risk compared to men (HR 1.32; 95%CI: 0.88-2.00). Compared to weight gain without metabolic dysfunction [metabolically healthy obesity; (MHO)], weight gain with metabolic dysfunction increases the overall risk of cancer risk by 77% (HR 1.77; 95%CI: 1.21-2.59), with men (HR 1.85: 95%CI: 1.00-3.44) having higher risk compared to women (HR 1.74; 95%CI: 1.07-2.82). The study also observed that men and women who gained weight during adult life from non-overweight status at baseline, were associated with 2.18-fold and 1.60-fold overall cancer risk, whereas those who were overweight throughout the study period (from baseline) were associated with statistically non-significant increased cancer risks of 28% (HR 1.28; 95%CI: 0.76-2.14) and 33% (HR 1.33; 95%CI: 0.94-1.88), in men and women, respectively[24].

Nearly 10%-30% of obese individuals are metabolically healthy with lesser visceral and hepatic fat, greater leg fat, expandable subcutaneous fat, preserved cardiorespiratory fitness, insulin sensitivity, and beta cell/adipose tissue function, and lower inflammatory burden[25]. Though there is no standard definition for MHO, presence of obesity with normal glucose and lipid parameters in the absence of hypertension can be used as a criterion to diagnose MHO. Though the risk for getting T2DM and cardiovascular disease is much lower in MHO people compared to people with metabolically unhealthy obesity (MUO), it is still higher than metabolically healthy lean (MHL) people. Moreover, MHO is a transient phenotype that can progress to develop MUO. Hence, MHO should still be considered as an indication for weight loss interventions[25]. A meta-analysis of eight prospective cohort studies comprising of 12542390 participants compared the incidence of any type of cancer in MHO people in comparison to people with metabolically healthy non-obesity (MHNO)[26]. They reported a significantly higher risk of developing cancer with an odds ratio (OR) of 1.14 (95%CI: 1.05-1.23) compared to MHNO people, and 1.17 (95%CI: 1.01-1.35) compared to MHL people. This suggests that all obese individuals, even in the absence of metabolic dysfunction, should be encouraged to lose weight.

A meta-analysis of 230 cohort studies including over 30 million individuals observed that, though overweight and obesity were associated with an increased risk of all-cause mortality, there was a U-shaped association[27]. The concept that cancer patients with elevated BMI might have improved survival compared to cancer patients with normal BMI is known as 'obesity paradox in cancer'. According to many, the term 'obesity paradox' is misleading as the paradox is due to the limitations of BMI, which relies on height and weight without delineating the distribution of adipocytes or distinguishing between adipose tissue and skeletal muscle. According to them, cancer patients with higher BMI might be having higher levels of protective skeletal muscle mass[28]. Others consider that, the paradox is due to methodological flaws including reverse causation, selection bias, and confounding[29]. However, a recent meta-analysis of 203 observational studies including 6320365 participants observed that even though obesity is associated with increased overall mortality, cancer specific mortality, and relapse rate in various cancers, it (obesity) is associated with an apparent protective effect in patients with lung cancer and melanoma[30].

Another meta-analysis of eight population-based cohort studies including 635642 participants who underwent bariatric surgery observed that, bariatric surgery is associated with a significantly reduced incidence of cancer (OR 0.72; 95%CI: 0.59-0.87) overall, and obesity-related cancer in particular (OR 0.55; 95%CI: 0.31-0.96)[31]. However, the reduction in incidence of breast cancer reached statistical significance (OR 0.50; 95%CI: 0.25-0.99), whereas reduction in other cancers did not reach statistical significance. A recent meta-analysis of 21 cohort studies comprising of 304516

participants who underwent bariatric surgery, revealed that bariatric surgery was not only associated with decreased cancer incidence (OR 0.56; 95%CI: 0.46-0.68), but also with decreased cancer mortality (OR 0.56; 95%CI: 0.41-0.75)[32]. The study also observed a significant reduction in breast and endometrial cancers in post-bariatric surgery participants.

Few observational studies reported a controversial observation about an increased incidence of colorectal cancer, in the post-bariatric surgery participants[33]. However, even in these trials, the absolute incidence of colorectal cancer was lower in the bariatric surgery group compared to the obese patients who did not undergo bariatric surgery. The cessation of statin therapy, avoidance of high fibre diet, and changes in colonic microbiome after bariatric surgery could explain a possible increase in the incidence of colorectal cancer in post-bariatric surgery cases.

A large study including 22198 participants who underwent bariatric surgery from the Kaiser Permanente Integrated health data reported a 33% reduction in any cancer incidence (HR 0.67; 95%CI: 0.60-0.74), and 41% reduction in adiposity-related cancer incidence (HR 0.59; 95%CI: 0.51-0.69)[34]. Among the adiposity related cancers, surgery is associated with a statistically significant reduction in postmenopausal breast (HR 0.58; 95%CI: 0.44-0.77), colon (HR 0.59; 95%CI: 0.36-0.97), endometrial (HR 0.50; 95%CI: 0.37-0.67), and pancreatic cancers (HR 0.46; 95%CI: 0.22-0.97), compared to obese patients who did not undergo bariatric surgery. Furthermore, a recent meta-analysis of seven studies including 1213727 participants observed that bariatric surgery reduces colorectal cancer by 36% (RR 0 ·64, 95%CI: 0.42-0.98)[35].

Epidemiological studies linking diabetes to cancer

Observational studies have consistently reported that people with T2DM have an increased risk for several types of cancers including liver, pancreas, endometrium, colorectal, breast, and bladder, and a decreased risk for prostate cancer. The observed association between T2DM and cancer could either be a causal (caused by hyperinsulinaemia or hyperglycaemia), or be a confounder (arising from common risk factors such as adiposity)[36]. The contributions from obesity and T2DM towards tumourigenesis can be independent as exemplified by prostate cancer, the incidence of which is increased with obesity, but decreased with T2DM. Another example is lung cancer, the incidence of which is lower in obesity, but not altered with T2DM. The contributions of obesity and T2DM towards cancer can have an additive (synergistic) effect or an opposing effect, depending on the site of origin of cancer[6].

An umbrella review of 'meta-analyses of observational studies that examined the association between T2DM and cancer' carefully assessed the robustness of the reported associations, considering the quality of the studies and their substantial heterogeneity[36]. The review observed that only a minority of these reported associations have evidence-base without hints of bias. These observed summary associations in the descending order of random effects include endometrial cancer (1.97; 95%CI: 1.71-2.27), intrahepatic cholangiocarcinoma (1.97; 95%CI: 1.57-2.46), colorectal cancer (1.27; 95%CI: 1.21-1.34), and breast cancer (1.20; 95%CI: 1.12-1.28).

A meta-analysis of forty-five observational studies comprising more than eight million participants and 132331 prostate cancer patients revealed a statistically significant inverse association between T2DM and carcinoma of prostate (RR 0.86; 95%CI: 0.80-0.92)[37]. One point supporting the lower incidence of cancer prostate in T2DM is the fact that some men with T2DM with/without obesity have lower androgen levels that results in reduced stimulation of androgen sensitive prostate cancer cells[38]. Another point supporting the lower incidence of cancer prostate is a lower circulating prostate-specific antigen levels seen in men with T2DM with high hemoglobin A1c and fasting blood glucose in the obese, and men with raised alanine transaminase levels which would delay the diagnosis of cancer prostate[39].

Among cancer patients with pre-existing diabetes mellitus, there is a 41% increase in all-cause mortality (HR 1.41; 95%CI: 1.28-1.55)[40]. A subgroup analysis showed increased all-cause mortality with cancers of endometrium (HR 1.76; 95%CI: 1.34-2.31), breast (HR 1.61; 95%CI: 1.46-1.78), and colorectum (HR 1.32; 95%CI: 1.24-1.41). Another meta-analysis on colorectal cancer patients with pre-existing diabetes mellitus observed that the all-cause mortality was increased by 17% (RR 1.17; 95%CI: 1.09-1.25), and cancer specific mortality by 12% (RR 1.12; 95%CI: 1.01-1.24), compared to colorectal cancer patients without diabetes mellitus[41]. Moreover, presence of pre-existing diabetes mellitus was associated with a 51% higher post-operative mortality (OR 1.51; 95%CI: 1.13-2.02) among cancer patients[42]. Cancer patients with pre-existing diabetes mellitus exhibited advanced stage of the disease at the time of diagnosis[43], increased risk of cancer recurrence[44], and decreased disease-free survival (RR 1.27; 95%CI: 1.06-1.52)[41].

However, we ought to bear in mind that observational epidemiological studies are susceptible to certain biases including reverse causality bias, detection bias, and depletion of the susceptible[45]. Mendelian randomization (MR) studies are analytic methods (genetic epidemiological studies) that are used to strengthen the evidence for a causal relationship between an exposure and an outcome. MR studies utilize germline variants obtained from large-scale genome-wide association studies. As these germline variants are determined at the time of birth and remain constant throughout life[46], studies utilizing them will minimize the effects of bias and residual confounding that are observed in observational studies. However, the genetic observational or MR studies have their own strengths and weaknesses. Once the observational and genetic epidemiological studies agree between each other, the results are likely to be more robust.

MR studies have shown that adiposity has a very strong causal association with renal, endometrial, ovarian, oesophageal, pancreatic, and colorectal cancer[46]. Hyperinsulinaemia has a strong association with endometrial, breast, pancreatic and renal cancer risk. Raised circulating insulin-like growth factor-1 (IGF-1) levels have a moderate association with breast and prostate cancer risk. Sex hormone dysregulation and puberty timing have a moderate association with breast and endometrial cancer risk; puberty timing has a moderate association with prostate cancer risk. There is only a weak association between hyperglycaemia and various cancers including those of lung, pancreas, endometrium, kidney, and breast. Finally, no association is observed between T2DM and cancers including pancreatic, endometrial, renal cell, and ovarian cancers[46].

POTENTIAL PATHOPHYSIOLOGICAL MECHANISMS LINKING OBESITY AND DIABETES TO CANCER

Direct effects of hyperinsulinaemia in the pathogenesis of cancer: Insulin receptor/IGF-receptor signalling

The increased incidence of various cancers including breast, endometrial, and colorectal cancers is observed within few months after the diagnosis of T2DM, or even in the prediabetic phase, indicating that in patients with T2DM, it is the endogenous hyperinsulinaemia, rather than hyperglycaemia, that is associated with an increased risk of cancer[47-50]. Moreover, in breast, colorectal and endometrial cancer patients, the endogenous hyperinsulinaemia is associated with cancer progression, recurrence, and excess mortality[51-54]. Compared to normal cells which preferentially rely on mitochondrial oxidative phosphorylation, the cancer cells rely on glycolysis, even in the presence of oxygen (aerobic glycolysis), as a source of energy, possibly due to damaged mitochondria in cancer cells and also as a measure to maximize the available energy sources to support the rapid proliferation. This observation, known as the Warburg effect, suggests an increased glucose uptake and increased reliance on glucose metabolism by the cancer cells[55].

Studies have shown that hyperglycaemia alone may not cause development of cancer in the absence of hyperinsulinaemia, indicating that the key driver of cancer initiation and progression in patients with diabetes, and obesity is hyperinsulinaemia[56]. However, there are multiple other mechanisms involved in cancer initiation and progression. The overall pathophysiological mechanisms linking obesity and diabetes to cancer and the associated intracellular signalling, are illustrated in Figure 1 and the pathophysiological mechanisms linking hyperin-sulinaemia in the tumour microenvironment (TME) to cancer, is represented in the Figure 2.

The insulin/IGF family consists of ligands including insulin, IGF-1, and IGF-2; their tyrosine kinase receptors including insulin receptor-A (IR-A), insulin receptor-B (IR-B), IGF-1 receptor (IGF-1R), IR-A/IGF-1R hybrid, and IR-B/IGF-1R hybrid; and six IGF-binding proteins (IGFBPs) that bind to IGF-1 and IGF-2, but not to insulin. Only the free IGFs, unbound to IGFBPs, are biologically available for binding to their receptors. As the IGFs bound to IGFBPs are protected from degradation, the IGFBPs maintain a stable serum IGF levels. Hyperinsulinaemia decreases IGFBP-1 and IGFBP-2 levels, thus increasing the levels of bioavailable IGF-1 and IGF-2[57]. Moreover, hyperinsulinaemia increases the IGF-1 level by increasing its hepatic production[58]. Apart from the IGFs that circulate in blood, a significant amount of IGF-2 is also secreted by cancer cells and/or tumour stroma to act on IR-A[59].

The IR signalling exerts both metabolic and mitogenic effects. Among the two isoforms that are formed by differential splicing of the insulin receptor gene (splice

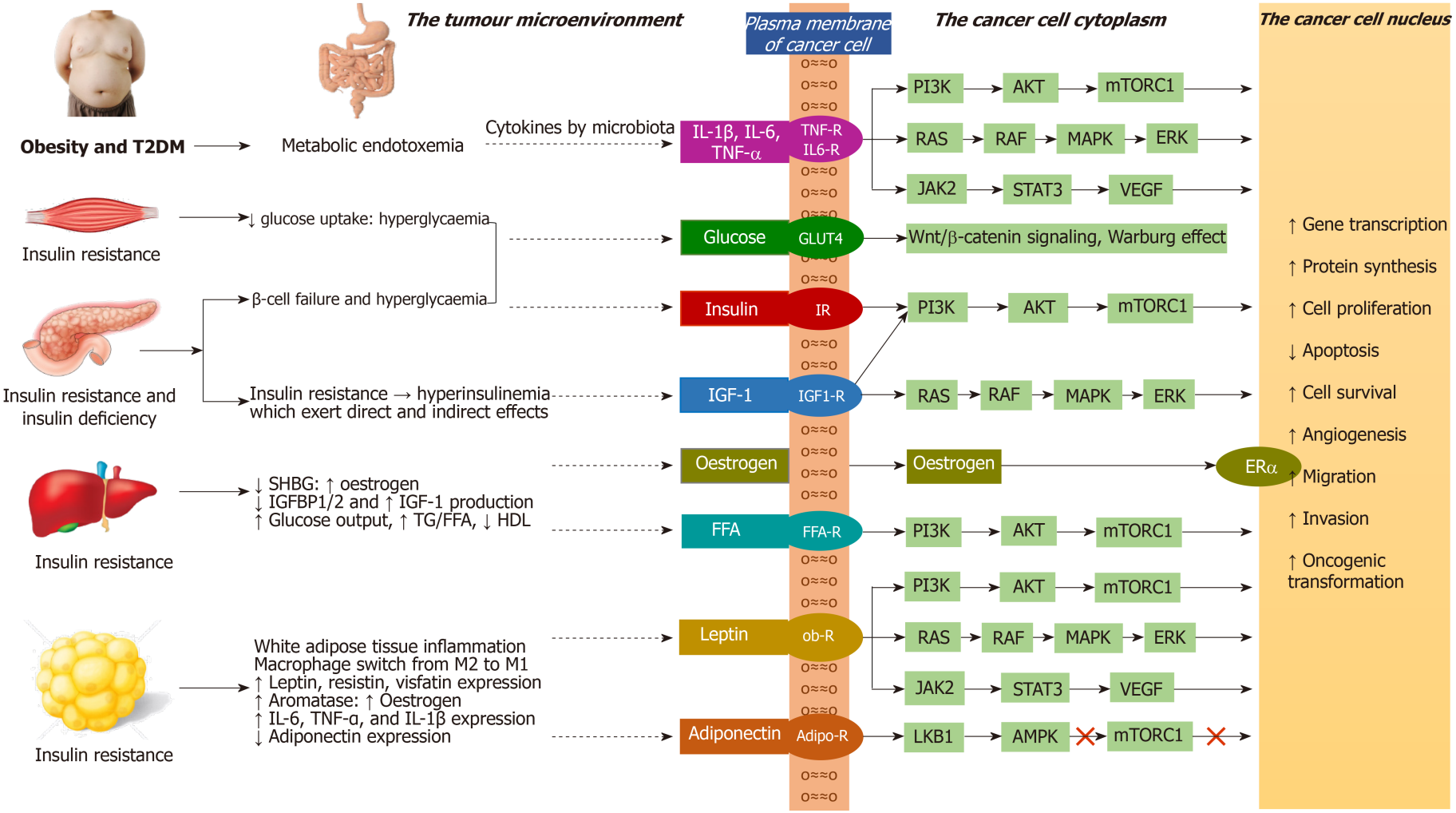

Figure 1 The overall pathophysiological mechanisms linking obesity and diabetes to cancer with associated intracellular signalling. IL-1β: Interleukin-1β; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-α; IR: Insulin receptor; IGF-1: Insulin-like growth factor-1; IGF-1R: Insulin-like growth factor-1 receptor; IGFBP: Insulin-like growth factor binding protein; FFA: Free fatty acid; FFF-R: Free fatty acid receptor; ER-α: Oestrogen receptor-α; Ob-R: Leptin-receptor; Adipo-R: Adiponectin-receptor; SHBG: Sex hormone binding globulin; TG: Triglyceride; HDL: High density lipoprotein; PI3K: Phosphatidyl-inositol-3-kinase; AKT: Protein kinase B; mTORC1: Mechanistic target of rapamycin complex 1 (Mammalian target of rapamycin complex 1); RAS: Rat sarcoma; RAF: Rapidly accelerated fibrosarcoma; MAPK: Mitogen activated protein kinase; ERK: Extracellular-regulated kinase; JAK2: Janus kinase-2; STAT3: Signal transducer and activator of transcription-3; VEGF: Vascular endothelial growth factor; HIF-1α: Hypoxia inducible factor-1α; LKB1: Liver kinase B1; AMPK: Adenosine monophosphate-activated protein kinase; T2DM: Type 2 diabetes mellitus.

variants), the IR-B is predominantly expressed in the metabolic tissues including liver, skeletal muscle, adipose tissue, and kidney, whereas IR-A is mainly expressed in the foetal and cancer tissues[60]. IR-B predominantly exerts metabolic effects, whereas IR-A predominantly exerts mitogenic effects. The ratio of IR-A to IR-B in the cell is determined by the expression of certain splicing factors in cells. Insulin, IGF-1, and IGF-2 bind to IR-A, and IR-B with different affinities. Insulin binds to IR-A with a 1.7-fold greater affinity compared to IR-B (only a modest difference in affinity). IGF-2 binds to IR-A with a 40-fold greater affinity compared to IR-B, whereas IGF-1 binds to IR-A with a 10-fold greater affinity compared to IR-B. Insulin binds only to IR-B or IR-A, not to IGF-1R or hybrid receptors. Both IGF-1 and IGF-2 bind to IGF-1R, hybrid receptors, and to IR-A or IR-B. IR-A has 100-fold higher affinity for IGF-2 compared to IGF-1[60]. Thus, IR-A has high affinity for IGF-2 and low affinity for IGF-1, whereas IR-B has a low affinity for IGF-2 and a very low affinity for IGF-1. High IR-A expression, resulting from altered expression of splicing factors in the cell is detrimental in adult life as it is associated with insulin resistance, dysregulated cell proliferation and cancer[61].

While normal cells downregulate the IRs in presence of hyperinsulinaemia, many cancer cells upregulate the IRs and IGF-1Rs in presence of hyperinsulinaemia and associated high IGF-1 levels, leading to mitogenic effects, increased cancer growth and metastasis[62,63]. Cancers that overexpress IR-A include breast, endometrial, lung, colorectal, hepatocellular, prostate, ovary, thyroid, and renal cancers[64-66]. Similarly, the cancers that overexpress IGF-1R include colorectal, breast, hepatocellular, and prostate cancers[67]. The loss of function mutations of tumour suppressor genes including *BRCA1*, *p53*, and *PTEN* lead to high IGF-1R expression[68]. Cancers that overexpress IGF-2 include mesenchymal tumours, breast, oesophageal, ovarian, and hepatocellular; tenosynovial giant cell tumours, Wilms' tumour, and Ewing's sarcoma[69].

Under physiological conditions (in people without hyperinsulinaemia), interaction of insulin and IR-B with subsequent stimulation of phosphatidyl-inositol-3-kinase/protein kinase B/mechanistic target of rapamycin complex 1 (PI3K/AKT/mTORC1) cascade mediate the anabolic effects of insulin including glucose uptake, glycogen synthesis, protein synthesis, and lipid synthesis. In people with hyperinsulinaemia (associated with high IR-A expression) and in cancer cells (associated with high IR-A expression and raised IGF-2), the interaction of insulin and/or IGF-2 with IR-A and the subsequent activation of rat sarcoma/rapidly accelerated fibrosarcoma/mitogen activated protein kinase/extracellular-regulated kinase cascade (RAS/RAF/MAPK/ERK) mediate the mitogenic effects of insulin including cell proliferation, survival, and migration[61]. An imbalance between MAPK and PI3K cascades results in impaired glucose/lipid metabolism in target tissues such as liver, muscle, and adipose tissue with cell proliferation in other tissues[70]. Under physiological conditions the interaction with IR-B is phasic (occurs only in postprandial state) resulting in metabolic effects, whereas under hyperinsulinaemic conditions or in cancer cells the interaction with IR-A is steady or continuous resulting in mitogenic effects[61].

Indirect effects of hyperinsulinaemia in the pathogenesis of cancer: Oestrogen receptor-α/cytokine/reactive oxidative species

Hyperinsulinaemia is associated with increased expression of aromatase enzyme in the TME resulting in increased oestrogen levels. Furthermore, hyperinsulinaemia is associated with decreased sex hormone-binding globulin levels that will increase the levels of bioavailable oestrogens that act on the tumour cells through oestrogen receptor-α, increasing the risk of oestrogen dependent cancers like breast and endometrial cancers[71]. The oestrogen receptor activation augments the insulin/IGF-mediated mitogenic effects in several cancers including that of breast, prostate, neuroblastoma, and pituitary adenoma[72].

Moreover, in carcinoma of prostate, activation of oestrogen receptors and of androgen receptors located at cell membrane induces IGF-1R upregulation to enhance IGF-1 mediated biological effects[73]. Similarly, in breast cancer, activation of IGF-1R and IR upregulate the non-classical or non-genomic membrane oestrogen receptors to potentiate the mitogenic effects[74,75]. Hyperinsulinaemia is also associated with inflammation in the TME leading to cytokine production and activation of the Janus Kinase-2 and Signal Transducer and Activator of Transcription-3 (JAK2-STAT3) and MAPK cascade inside the tumour cells[71]. Insulin upregulates the cellular metabolic activity leading to generation of reactive oxidative species (ROS) and resultant DNA damage, thereby promoting oncogenesis[76].

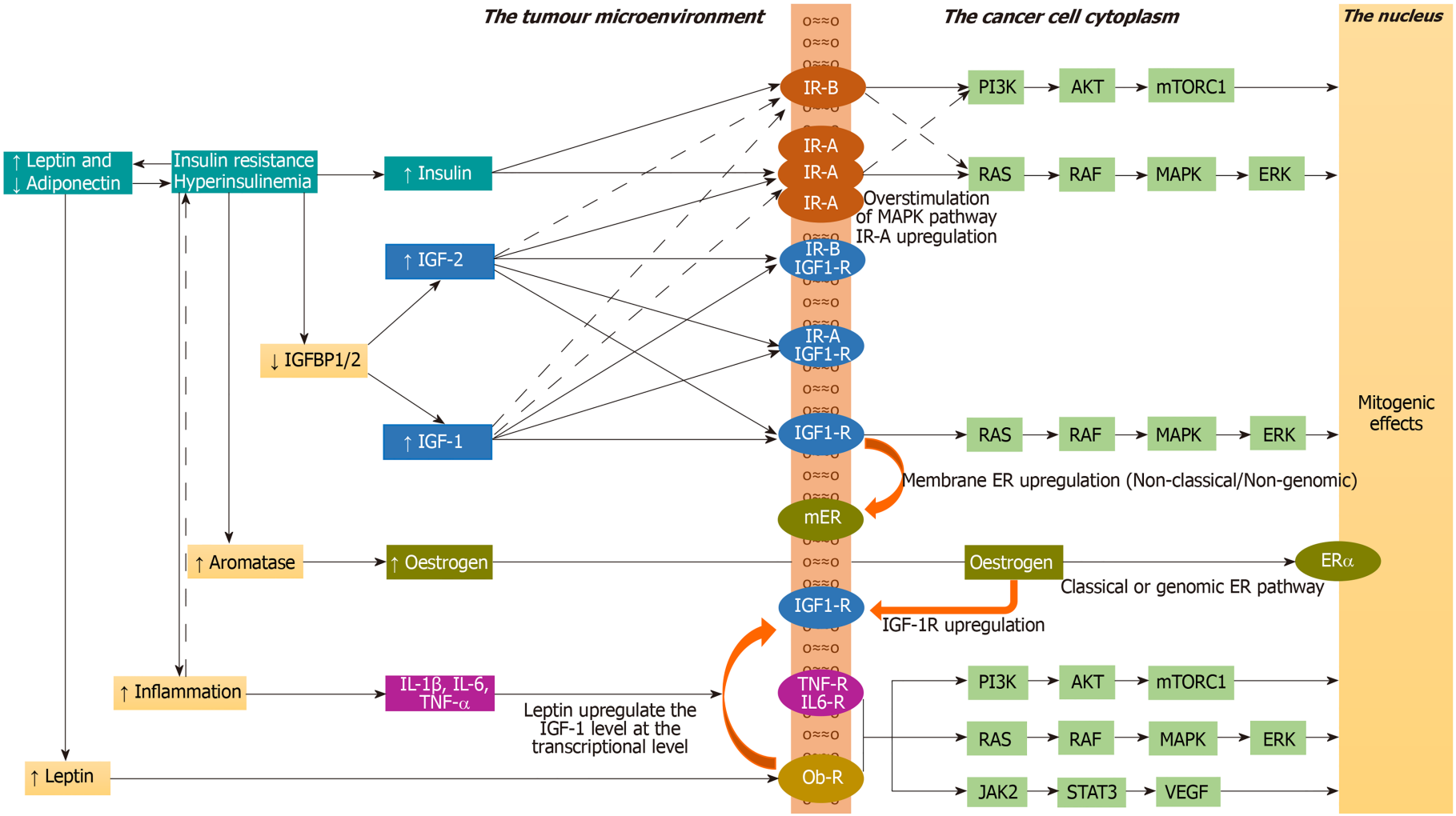

Figure 2 The pathophysiological mechanisms linking the hyperinsulinaemia in the tumour microenvironment to cancer with the associated intracellular signalling. IL-1β: Interleukin-1β; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-α; IR-A: Insulin receptor-A; IR-B: Insulin receptor-B; IGF-1: Insulin-like growth factor-1; IGF-2: Insulin-like growth factor-2; IGF-1R: Insulin-like growth factor-1 receptor; IR-A IGF1-R: Hybrid receptor of IR-A and IGF-1R; IR-B IGF1-R: Hybrid receptor of IR-B and IGF-1R; IGFBP: Insulin-like growth factor binding protein; ER-α: Oestrogen receptor-α; mER: membrane oestrogen receptor; Ob-R: Leptin-receptor; PI3K: Phosphatidyl-inositol-3-kinase; AKT: Protein kinase B; mTORC1: Mechanistic target of rapamycin complex 1 (Mammalian target of rapamycin complex 1); RAS: Rat sarcoma; RAF: Rapidly accelerated fibrosarcoma; MAPK: Mitogen activated protein kinase; ERK: Extracellular-regulated kinase; JAK2: Janus kinase-2; STAT3: Signal transducer and activator of transcription-3; VEGF: Vascular endothelial growth factor.

White adipose tissue remodelling in the pathogenesis of cancer

The white adipose tissue (WAT) comprising of subcutaneous and visceral adipose tissues, act as an energy reservoir for other organs. In response to over-nutrition and obesity, the adipose tissue undergoes dynamic remodelling characterized by alterations in the adipocyte number (adipocyte hyperplasia) in cases of childhood obesity or size (adipocyte hypertrophy) in cases of adult obesity[77]. The potential pathophysiological mechanisms linking obesity to cancer with special emphasis to WAT remodelling is outlined in the Figure 3. The hypertrophic adipose tissue outgrows its blood supply, leading to hypoxia, adipocyte injury/death, adipose tissue macrophage recruitment and a switch from anti-inflammatory to pro-inflammatory macrophages (M2 to M1 switch)[78]. This leads to increased expression of pro-inflammatory cytokines including tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1β and monocyte chemoattractant protein-1 (MCP-1), and insulin resistance[79].

Pro-inflammatory cytokines propagate the adipose tissue inflammation by recruiting more macrophages (MCP-1)[80]. The macrophages envelope the dead or dying hypertrophic adipocytes to form crown-like structures and these macrophages later become lipid-loaded foam cells[81]. There is increased release of free fatty acids (FFA) from the entrapped adipocytes with subsequent ectopic fat deposition in the liver and skeletal muscle leading to worsening insulin resistance and lipotoxicity[82]. Lipolysis and FFA release from WAT are also stimulated by pro-inflammatory cytokines[83]. The hypertrophic adipocytes exhibit impaired insulin-dependent glucose uptake due to a defect in glucose transporter 4 trafficking, indicating another mechanism for insulin resistance in obese patients with adipocyte hypertrophy, apart from the effect of pro-inflammatory cytokines and ectopic lipid deposition[84].

Pro-inflammatory cytokines generated by chronic low-grade inflammation of WAT can exert direct mitogenic effects *via* cytokine receptors or indirect mitogenic effects *via* increased insulin resistance and resultant hyperinsulinaemia. Moreover, the cytokines can activate androgen receptors to promote survival and proliferation of prostate cancer cells in men[85], and can induce the aromatase enzyme to increase the incidence of oestrogen-dependent tumours in the postmenopausal women[86]. The hyperleptinaemia that accompanies WAT inflammation is another inducer of aromatase enzyme[87].

Obesity and hyperinsulinaemia are associated with raised leptin and reduced adiponectin levels. Similarly, hyperleptinaemia and hypoadiponectinaemia are associated with the development of insulin resistance and hyperinsulinaemia[88,89]. The elevated leptin levels activate various cascades like PI3K, MAPK, and predominantly JAK2/STAT3[90]. Leptin induces IL-6 and TNF-α production, thereby sustaining a chronic inflammatory state[91]. It increases the expression of anti-apoptotic proteins (X-linked inhibitor of apoptosis protein), and pro-angiogenic factors including vascular endothelial growth factor (VEGF), and hypoxia-inducible factor-1α (HIF-1α)[92]. On the other hand, adiponectin, acting *via* liver kinase B1 (LKB1), induces the adenosine monophosphate-activated protein kinase (AMPK) involved in the induction of cell cycle arrest and inhibition of mTOR activity. Elevated leptin and decreased adiponectin levels are known to be associated with proliferation, survival and migration of cancers including that of breast, colon, endometrium, and prostate[92].

Hypoxia and angiogenesis in the pathogenesis of cancer

The hypertrophic adipose tissue outgrows its blood supply and develops hypoxia. HIF expressed in the hypoxic TME is a dimeric transcription factor having inducible subunits (HIF-1α, HIF-2α, or HIF-3α), and a constitutive subunit (HIF-1β)[93]. Hypoxia stabilizes HIF-1α and promotes its association with HIF-1β. The HIFα-HIFβ dimer enters the nucleus leading to activation of the downstream targets. Under hypoxic conditions, HIF-1α promotes tumour angiogenesis by activating the pro-angiogenic genes [(*VEGFA*, *VEGF receptor-1* (*VEGFR1*), *Angiopoietin* (*ANGPT*), and *Ephrin type-A receptor 1* (*EphA1*)], and inhibiting anti-angiogenic genes (*VEGFA*, *VEGFR1*, *ANGPT*, *EphA1*)[93]. Tumour angiogenesis is essential for the survival, growth, invasion, and metastasis of malignant lesions.

Oxidative stress in the pathogenesis of cancer

The metabolically active adipose tissue is a source of ROS/reactive nitrogen species. The adipose tissue from lean individuals expresses antioxidant enzymes including glutathione peroxidase, catalase, and superoxide dismutase 1, whereas these antioxidant enzymes are downregulated in the adipose tissue from obese individuals[83]. The oxidative stress is known to cause DNA double strand breaks and other complex DNA alterations[94]. Low or intermediate levels of oxidative stress

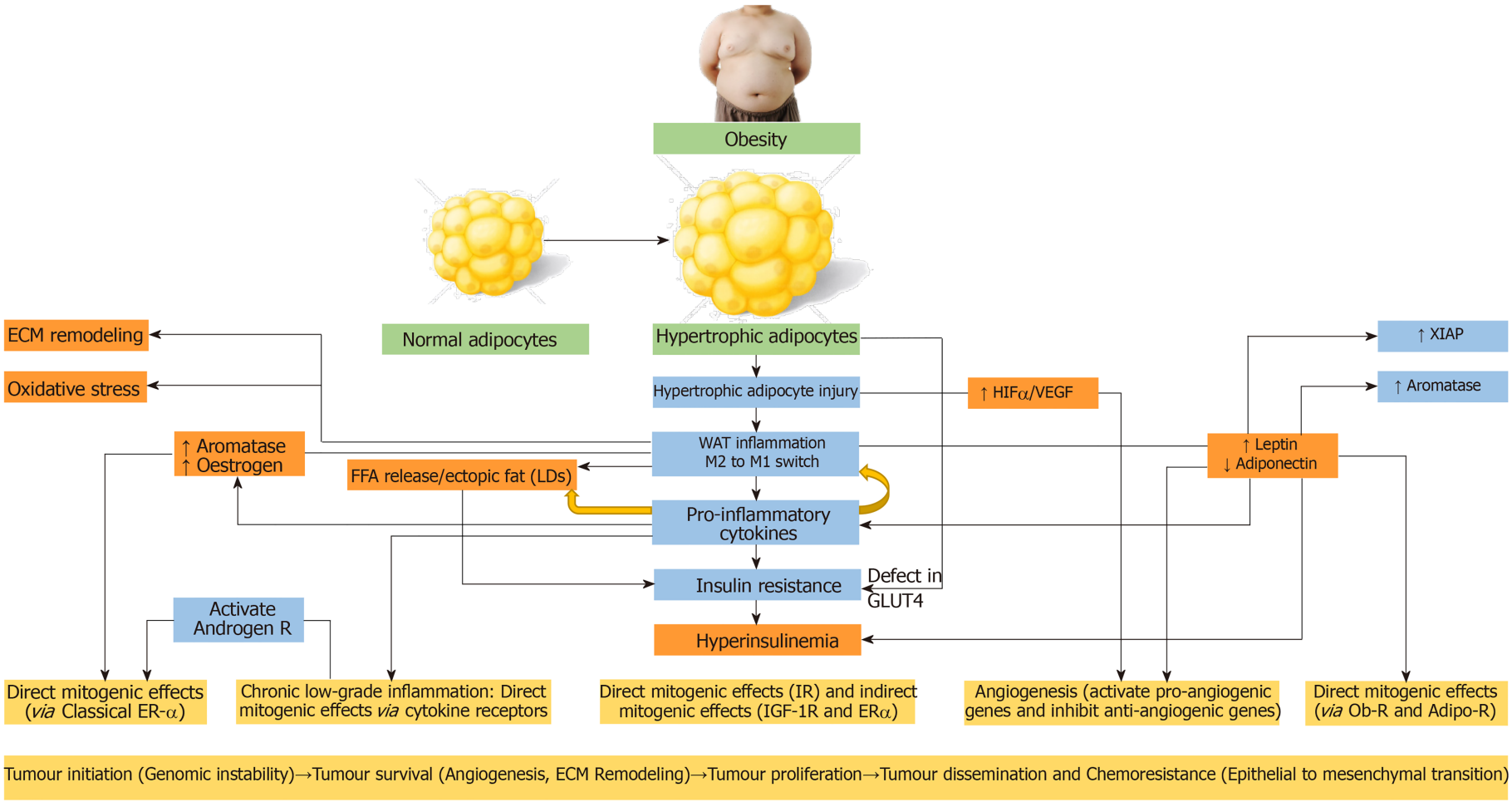

Figure 3 The potential pathophysiological mechanisms linking obesity to cancer with special emphasis to the white adipose tissue remodelling. WAT: White adipose tissue; IR: Insulin receptor; IGF-1R: Insulin-like growth factor-1 receptor; ER-α: Oestrogen receptor-α; Ob-R: Leptin-receptor; Adipo-R: Adiponectin-receptor; FFA: Free fatty acid; LD: Lipid droplets; ECM: Extracellular matrix; XIAP: X-linked inhibitor of apoptosis protein; VEGF: Vascular endothelial growth factor; HIF-1α: Hypoxia inducible factor-1α; GLUT4: Glucose transporter 4.

result in genomic instability associated with the activation of oncogenes, inactivation of tumour suppressor genes, angiogenesis, and mitochondrial dysfunction[95]. Obesity *per* se is associated with increased DNA damage and decreased DNA repair. Oxidative

stress can be a consequence of obesity. Moreover, oxidative stress can be the trigger for obesity by altering the food intake and stimulating WAT deposition[96-98].

In obesity associated WAT inflammation, the inflammatory environment increases the oxidative stress to a level that it results in DNA damage, genomic instability, augmented cell survival, and cell proliferation resulting in the development of cancer[99]. Increased ROS production has been observed in various cancers. Tumour cells express high levels of antioxidants to detoxify ROS, to establish a redox balance while maintaining a resistance to apoptosis. Though ROS can be pro-tumourigenic in most, they can also be anti-tumourigenic, initiating tumour cell death, especially when the ROS levels exceed the antioxidant threshold of cancer cells[100].

Extracellular matrix alterations in the pathogenesis of cancer

The TME comprises of a cellular and a non-cellular component. The cellular component includes immune cells, fibroblasts, adipocytes, and endothelial cells, whereas the non-cellular structural component, known as the extracellular matrix (ECM) include a meshwork of polymeric proteins like collagen, elastin, and fibronectin. The ECM provides the biochemical and biomechanical environment within which the cancer cells exist[101]. WAT inflammation induces mechanical changes in the ECM, including myofibroblast enrichment with associated increased stiffness that promote tumourigenesis[102]. Moreover, crosstalk between cancer cells and the microenvironment is an important aspect of tumour progression, as this determines the ability of cancer cells to cross the ECM barrier, access the circulation, and establish metastases[103]. The biochemical and biomechanical properties of the ECM influence the ability of the cancer cells to modify physiological features (plasticity) to survive in the hostile microenvironment, and to resist therapy through acquisition of stemness characteristics and epithelial to mesenchymal or mesenchymal to epithelial transitions[103,104].

Resistin, visfatin, and lipid droplets in the pathogenesis of cancer

The obesity associated chronic low-grade inflammatory state in the adipose tissue, results in genomic instability contributing to tumourigenesis. Moreover, obesity is associated with aggressive cancers, due to the crosstalk between adipose tissue and tumours during cancer progression[105]. The mature adipocytes supply adipokines and lipids to the proliferating cancer cells, whereas the adipose stromal cells, and the immune cells infiltrate the tumour tissue to secrete various paracrine factors within the TME to aid tumour progression. Presence of high levels of leptin and/or leptin-receptor is associated with poor prognosis in several cancers as evidenced by the presence of invasive tumours, lymph node involvement, distant metastasis, and chemoresistance[106]. Elevated leptin levels can upregulate the IGF-1 level acting at the stage of transcription[107]. Resistin and visfatin acts through their receptors to promote tumour cell proliferation, angiogenesis, metastasis, and chemoresistance[108,109].

Obesity is associated with ectopic fat deposition containing FFAs, triglycerides and cholesterol esters in non-adipose tissues. These lipid bodies, known as lipid droplets (LDs), are seen in many cancers, where they are thought to modulate the crosstalk between tumour cells and the cellular component of the TME. LDs are associated with tumour proliferation, chemoresistance, and aggressiveness[110]. Recently, fatty acid receptors with selectivity towards medium-long chain fatty acids (FFAR4 and FFAR1), and towards short chain fatty acids (FFAR2 and FFAR3) are discovered. FFAR4 is associated with proliferation, survival and migration of various cancers including colorectal, pancreatic and bone cancers[111]. The FFAs mediate the proliferation and metastasis of the tumour cells by activating the PI3K-AKT-mTORC1 pathway[112].

Hyperglycaemia in the pathogenesis of cancer

There are many mechanisms that can contribute to high cancer risk in patients with diabetes. The potential mechanisms, with a special emphasis to the Wnt/β-catenin signalling pathway, are portrayed in the Figure 4. These mechanisms can be related to antidiabetic medications[113], hormonal changes (exogenous or endogenous hyperinsulinaemia, raised IGF-1, hyperleptinaemia, and hypoadiponectinaemia), chronic inflammatory state associated with diabetes, oxidative stress associated with diabetes, decreased immunological response to cancer cells arising from competitive impairment of ascorbic acid transport into the immune cells by hyperglycaemia[114], enhanced signalling of epidermal growth factor receptor[115], accelerated cell cycle[116], chemoattractant upregulation, such as glial cell line-derived neurotrophic factor that is involved in the cancer invasiveness and migration[117], cytokine receptor upregulation and ROS generation by the advanced glycation end products

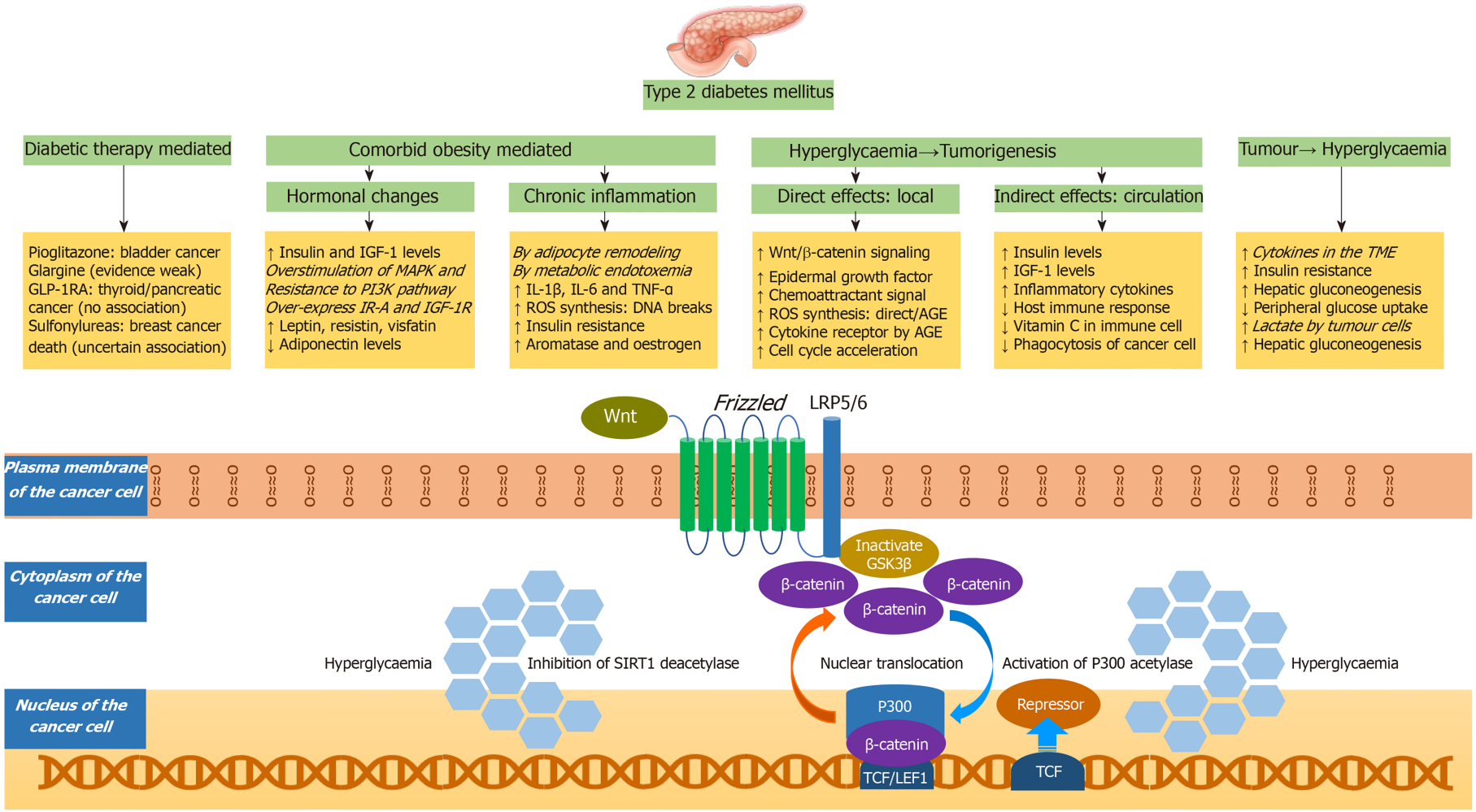

Figure 4 The potential pathophysiological mechanisms linking diabetes to cancer with special emphasis to the Wnt/β-catenin signalling pathway. IGF-1: Insulin-like growth factor-1; IGF-1R: Insulin-like growth factor-1 receptor; IL-1β: Interleukin-1β; IL-6: Interleukin-6; TNF-α: Tumour necrosis factor-α; IR-A: Insulin receptor-A; ROS: Reactive oxygen species; GLP-1RA: Glucagon like peptide-1 receptor agonist; MAPK: Mitogen activated protein kinase; PI3K: Phosphatidyl-inositol-3-kinase; AGEs: Advanced glycation end products; TME: Tumour microenvironment; LRP: Lipoprotein receptor-related protein; GSK3β: Glycogen synthase kinase-3β; SIRT1: Sirtuin 1 deacetylase; P300: P300 acetyl transferase; LEF: Lymphoid enhancer factor; TCF: T-cell factor.

(AGEs)[118], and most importantly enhanced Wnt/β-catenin signalling pathway resulting in increased proliferation, survival, invasion, and migration[119]. The raised insulin and IGF-1 levels are associated with overstimulation of MAPK pathway, resistance to PI3K pathway, over-expression of IR-A and activation of IGF-1R[120]. The oxidative stress associated with diabetes can occur through multiple mechanisms: direct effect of hyperglycaemia through glucose metabolism and auto-oxidation, or indirect effect from AGEs, or inflammatory cytokines[120].

Wnt is a family of secreted cysteine-rich glycoprotein ligands that bind to their membrane receptors to activate pathways including non-canonical Wnt-Ca^{2+} pathway, non-canonical planar cell polarity pathway, and canonical Wnt/β-catenin signalling pathway[121]. The classification of Wnt family into canonical or non-canonical is based on the presence or absence of β-catenin. In the canonical Wnt/β-catenin pathway, Wnt binds to its membrane co-receptor having Frizzled and lipoprotein receptor-related protein. This inactivates the Glycogen Synthase Kinase-3β (GSK3β), resulting in β-catenin accumulation in the cytoplasm. GSK3β is an enzyme that phosphorylates the cytosolic β-catenin to trigger degradation of β-catenin by the destruction complex. GSK3β is thereby considered as a tumour suppressor, due to its ability to inhibit the Wnt/β-catenin signalling pathway[122].

In the absence of hyperglycaemia, β-catenin accumulated in the cytoplasm cannot be translocated to the nucleus, to induce the expression of Wnt target genes. However, hyperglycaemia induces p300 acetyl transferase to achieve β-catenin acetylation. Moreover, hyperglycaemia inhibits Sirtuin 1 deacetylase activity. These favour formation of lymphoid enhancer factor 1 (LEF1)/β-catenin/p300 complex and its accumulation inside the nucleus, where it displaces the transcriptional repressor known as T-cell factor (TCF)7L2-corepressor complex, and induce the expression of Wnt target genes (LEF, TCF)[118,123]. These Wnt target genes are involved in initiation, proliferation, senescence bypass, epithelial to mesenchymal transition, and metastasis of tumours[124-127].

Cancer worsens hyperglycaemia

In patients with cancer, the circulating cytokines increases the insulin resistance, decreases the peripheral glucose uptake, increases the hepatic gluconeogenesis, thereby worsens hyperglycaemia. Increased inflammatory cytokines in the TME worsens this hyperglycaemia. Moreover, the product of glycolysis by tumour cells (lactate) stimulates the hepatic gluconeogenesis, further worsening the hyperglycaemia[119]. A recently published study from Korea has shown that cancer can increase the risk of getting subsequent diabetes mellitus in cancer survivors independent of traditional risk factors for diabetes mellitus (HR 1.35; 95%CI: 1.26-1.45)[128]. Though the risk was highest in the first 2 years, it remained high for 10 years following cancer diagnosis (HR 1.19; 95%CI: 1.00-1.43). Though the risk was highest for cancer survivors of pancreatic, kidney, and liver cancers, the risk remained significantly high even for gallbladder, lung, blood, breast, stomach, and thyroid cancers.

Therapeutic strategies for cancer based on the pathophysiological mechanisms

The therapeutic agents based on Wnt/β-catenin signalling include those that act by inhibiting Wnt ligands, inhibiting Wnt receptors/co-receptors, stabilizing the destruction complex, and inhibiting β-catenin-dependent transcriptional pathway[129]. Moreover, GSK3β inhibitors are being developed and entering clinical trials as novel cancer treatments due to their ability to inhibit the Wnt/β-catenin signalling pathway[130]. mTOR participates in multiple signalling pathways to regulate proliferation, autophagy, and apoptosis. Various newly developed mTOR inhibitors are entering clinical studies[112]. The free fatty acid receptors agonists are potential therapeutic agents in the management of cancers of colorectum, and ovary[131,132].

Various inhibitors of MAPK signalling pathway including RAS inhibitors, RAF inhibitors, MAPK inhibitors, and ERK inhibitors have also been recently developed[133]. Three RAF inhibitors and three MAPK inhibitors have received approval for the treatment of late-stage B-RAF harbouring cancers, either alone or in combination with other agents. However, these drugs are associated with intrinsic drug resistance in patients with RAS mutations or acquired drug resistance in patients with B-RAF mutations (after 6-10 mo of treatment). Targeting MAPK and AMPK signalling pathways together represents a promising therapeutic intervention in patients with RAS or RAF mutations[134]. Another promising intervention in patients with B-RAF mutation-associated cancers, is dual inhibition of the MAPK and JAK2/STAT3 pathways using a combination of three MAPK pathway inhibitor types

including BRAF inhibitor, MAPK inhibitor, and ERK inhibitor along with either of JAK2 or STAT3 inhibitor[135].

Though PI3K signalling pathway is important in cell proliferation, and survival, the drugs acting on this pathway, including pan-PI3K inhibitors or dual PI3K/mTOR inhibitors are only modestly effective as monotherapy, with a relatively high incidence of side effects. However, isoform selective PI3K inhibitors are undergoing clinical trials with improved specificity and reduced toxicity[136]. Similarly, several AKT inhibitors are currently in various stages of clinical trials for diverse types of malignancies[137]. AMPK acts as tumour suppressor, as it mediates the effects of the LKB1 tumour suppressor by inhibiting mTORC1 production. Though metformin, and fluoxetine can activate AMPK, several small molecular AMPK agonists are under various stages of development and few of them are expected to enter clinical trials within next few years[138].

As hyperinsulinaemia is the key driver of cancer initiation and progression in patients with diabetes and obesity, drugs that could reduce hyperinsulinaemia could potentially prevent development of cancer. At supra-physiological concentrations, metformin can exert direct anti-proliferative effects. However, at physiological concentrations the anti-proliferative effects are due to its indirect effects including reduction in hyperglycaemia, insulin, IGF-1, and leptin[139]. Clinical trials with the use of metformin in cancer therapy and prevention are ongoing. Peroxisome proliferator-activated receptor gamma (PPARγ) is expressed in cancers including breast, prostate, colon, bladder, and thyroid cancers. Preclinical trials have shown that the PPARγ agonists have tumour suppressor effect as they are pro-apoptotic, induce autophagy, decrease cancer cell invasion and metastatic potential. However, the results of these clinical trials are disappointing due to their side effect profiles[140].

CONCLUSION

Obesity and T2DM are associated with high risk of cancer, and the strongest associations are for postmenopausal breast and endometrial cancers, and colorectal carcinomas. Mendelian randomization studies have shown that obesity and hyperinsulinaemia have very strong associations with cancer, whereas hyperglycaemia and T2DM have either a weak, or no association with cancer. The relationship between T2DM and cancer is bidirectional, as cancer survivors appear to be susceptible to subsequent new onset diabetes mellitus. Optimal screening strategies for diabetes in cancer survivors should be developed. With the increasing global burden of obesity and diabetes mellitus, the burden of cancer will continue to rise in the coming decades. Interventions at all possible levels, should be done to prevent the development of cancer from these common non-communicable diseases. Pathophysiological studies have shown that hyperinsulinaemia has the primary role in tumourigenesis in the setting of obesity and diabetes, associated with chronic inflammation, and elevated adipokines. In addition, patients with diabetes mellitus exhibit enhanced Wnt/β-catenin signalling pathway as one of the possible pathophysiological mechanisms. Newer therapeutic agents based on pathophysiological mechanisms including Wnt/β-catenin, MAPK, PI3K, AMPK and mTOR signalling pathways are undergoing preclinical/clinical trials for the treatment of cancer.

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REVIEW

Molecular diagnosis in cat allergy

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Abstract

Domestic cats represent one of the most common sources of indoor allergens. All over the world, many households own cats, whose allergens are persistent and widespread. Cat allergy itself is frequent, and its symptoms vary from rhinoconjunctivitis to life-threatening asthma. *In vitro* diagnosis using precision medicine allergy immunoassays is important because natural cat dander extracts may differ in quality and quantity of some of the individual allergen components and other molecules. In the component-resolved diagnosis of cat allergy, singleplex and multiplex specific immunoglobulin (Ig) E assays include use of the cat-specific major allergen, secretoglobin Fel d 1 (as a species-specific molecule), other allergen components (such as lipocalins Fel d 4, cross-reacting with other animal similar molecules, and Fel d 7, present in small quantities in natural extracts), and serum albumin Fel d 2 (related to the cat-pork syndrome). IgA Fel d 5 and IgM Fel d 6 are not available as allergen components in the current commercial IgE immunoassays, but they may impair the *in vitro* diagnostic evaluation of cat allergy because galactose-α1,3-galactose is an IgE-binding epitope of these native feline allergens. The benefits of molecular-based cat allergy diagnosis are continually evaluated, as the role of recombinant allergen components already known is detailed and new other molecules of interest may be discovered in the future.

Key Words: Feline; Allergens; Component-resolved diagnosis; Immunoglobulin E; Immunoassays

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Core Tip: Cats are a common source of allergens for humans, and allergy to these pets are frequent and variable in their clinical manifestations. The benefits of molecular diagnosis in cat allergy include use of the species-specific major allergen Fel d 1, cross-reacting allergen components, including those present in small quantities in natural extracts, while considering molecules that may impair the *in vitro* allergy diagnosis. The identification and characterization of molecular cat allergens with clinical significance has allowed their use in singleplex and multiplex immunoglobulin E immunoassays for a precision diagnostic approach.

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INTRODUCTION

The domestic cat (*Felis domesticus*, synonym: *Felis catus*) is one of the most common sources of indoor allergens, and allergy to cats in humans is the most common mammalian-origin immunoglobulin (Ig) E-mediated hypersensitivity. Cats have been associated with humans for more than 9500 years and are considered nowadays the most popular pets in the world. In past decades, a high incidence of allergy to these furry animals, especially among children and young adults, has been recorded. Cat allergy is currently estimated to affect approximately 1 in 5 adults worldwide. Many households own cats, indicating that there is a high exposure to their allergens. Moreover, the major and most studied cat allergen, Fel d 1, is persistent and ubiquitously present in indoor habitation spaces, dust samples from homes with or without cats, in public buildings and transportation, making allergen avoidance difficult[1-3]. The symptoms of allergy to cats vary from relatively mild rhinoconjunctivitis to potentially life-threatening asthma exacerbations[2].

Precision medicine allergy immunoassays support the molecular-based diagnosis for cat allergy. Also known as component-resolved diagnostics (CRD), this patient IgE sensitization *in vitro* molecular-level diagnostic approach uses allergenic components.

To date, eight *Felis domesticus* molecular allergens have been recognized as Fel d 1 to Fel d 8 by the World Health Organization/International Union of Immunological Societies (WHO/IUIS)[4]: uteroglobin-like protein Fel d 1, serum albumin Fel d 2, cystatin Fel d 3, lipocalins Fel d 4 and Fel d 7, Igs Fel d 5 and Fel d 6, and latherin-like protein Fel d 8. Cat allergens are involved in the molecular mechanisms underlying IgE-mediated allergic sensitization and different cross-reactivities. Representative isoforms are described for these allergens: Fel d 1.0101, Fel d 2.0101, Fel d 3.0101, Fel d 4.0101, Fel d 5.0101, Fel d 6.0101, Fel d 7.0101, Fel d 8.0101, but none is mentioned as such in the commercial IgE immunoassays. Data on the IgE binding epitopes are scarce, with sequence positions mentioned only for Fel d 1. IgE epitope mapping of this dominant cat allergen revealed five sequential/linear epitopes on chain 1/Fel d 1-A and two on chain 2/Fel d 1-B, in addition to a discontinuous/conformational epitope on chain 1[5], the last one being located on the four helices of the Fel d 1 chain 1 spatially juxtaposed upon protein folding.

Currently, the best characterized and available cat allergenic molecules for commercial IgE assays are Fel d 1, Fel d 2, Fel d 4 and Fel d 7. The two types of such allergen components used in singleplex and multiplex immunoassays are recombinant (r) allergens (produced by recombinant DNA technology) and highly purified natural (n) allergens (purified from natural sources)[6]. All are included in the list of cat allergens presented in the European Academy of Allergy and Clinical Immunology Molecular Allergology User's Guide[7] and in a recent Consensus document on dog and cat allergy[8]. The characteristics of these cat allergens[7-11] are presented in Table 1 together with all other allergenic molecules recognized by the WHO/IUIS database[4].

One major advantage of the CRD is the evaluation of primary sensitization animal source, which is not feasible by using native extracts, and better management of pet

Table 1 Characteristics of cat molecular allergens[7-11] mentioned in the World Health Organization/International Union of Immunological Societies database[4]

Allergen	Biochemical designation	Source of exposure	MW in kDa
Fel d 1[1,2]	Secretoglobin[4]	Saliva, dander	38
Fel d 2[1,3]	Serum albumin	Dander, serum, urine	69
Fel d 3	Cystatin[5]	Dander	11
Fel d 4[1,2]	Lipocalin[5]	Saliva	22
Fel d 5	Immunoglobulin A[4]	Saliva, serum	400
Fel d 6	Immunoglobulin M[4]	Saliva, serum	800-1000
Fel d 7[2]	Lipocalin, von Ebner gland protein	Saliva	17.5
Fel d 8	Latherin-like protein	Saliva	24

The World Health Organization/International Union of Immunological Societies is commonly known by its acronym, WHO/IUIS. Fel d 1, Fel d 2, Fel d 4 and Fel d 7 allergens are available in commercial immunoglobulin E immunoassays:
[1]Available in singleplex immunoassays as recombinant allergen.
[2]Available in multiplex immunoassays as recombinant allergen.
[3]Available in multiplex immunoassays as native purified component.
[4]Presence of glycosylation.
[5]Glycosylation deduced from sequence analysis. MW: Molecular weight.

allergic patients[1]. The importance of molecular-based diagnosis is continually evaluated, as the role of allergen components already identified in cat allergy is detailed, and new molecules of interest may be discovered.

CAT ALLERGEN COMPONENTS FOR MOLECULAR DIAGNOSIS

A deep understanding of the most important cat allergens is crucial for assessing allergen products for *in vitro* molecular diagnosis to evaluate in detail the IgE sensitization profile of patients allergic to furry pets. Other allergen proteins, recently identified and defined, must also be discussed for their potential use in CRD in the future.

Fel d 1

The cat major allergen Fel d 1 is a small tetrameric protein composed of two heterodimers, each containing two distinct chains (chain 1, a polypeptide, and chain 2, a glycopeptide with N-linked oligosaccharide composed of triantennary glycans) linked by disulfide bonds in its native form. This allergen is a secreted globular protein belonging to the secretoglobin family. It is homologous with the human Clara cell 10-kDa phospholipid-binding protein and the progesterone-binding rabbit uteroglobin (uteroglobinlike protein). Fel d 1's biological function for the cat is not clearly established, initially being discussed that it may have a protective role in cat skin[12-16]. Fel d 1 is probably involved in immunoregulation and intra-species chemical communication, binding with good affinity to some fatty acids and steroids, the best ligands being lauric acid (cat pheromone with effects on social interactions) and androsterone (volatile steroid pheromone). Fel d 1 is a thermostable protein produced in various anatomical areas of cats, mainly by the sebaceous glands and anal sacs, but also by salivary and lacrimal glands. Fel d 1 is primarily found in cat skin and hair follicles. As cats groom, Fel d 1 is distributed on the fur, then shed with hair and dander. It is easily airborne and found in various indoor environments, such as homes with and without cats, hotels, schools, buses and trains, occupational and/or leisure environments, including cinemas, animal facilities, pet shops, farms. Pet owner's clothing is a significant source of allergen dispersal. Up to 60% of airborne Fel d 1 molecules are carried by small particles, of which 75% are more than 5 μm in diameter and 25% less than 2.5 μm. This allergen is very pervasive indoors, many airborne Fel d 1 settles out within a couple of days of disturbance, but smaller particles can remain airborne for up to two weeks or even longer. Measurement of this secretoglobin allergen levels in settled dust should not be used as a surrogate for airborne exposure. Moreover, the concept of a specific allergen threshold amount of exposure expected to

provoke respiratory symptoms (such as 8 μg/g of dust) is also probably misleading, mentioning besides that IgE sensitization can occur at much lower Fel d 1 levels [1,3,12,16].

All cats produce Fel d 1 regardless of age, sex, breed, body weight, hair length or housing (indoors *vs* outdoors). Fel d 1 is produced under testosterone control (male cats produce more Fel d 1 than females if uncastrated and 3-5 times less after neutering, while its production could be restored to pre-neutering levels with exogenous testosterone administration)[1]. In the fur of domestic cats, Fel d 1 levels are significantly higher than those of Fel d 4, and cat-to-cat variability was revealed. The quantity of Fel d 1 on cat hair can range from 1 μg/g to more than 1770 μg/g, with high concentrations on hair from the neck region. The hair length does not seem to affect Fel d 1 production. Fel d 1 is also present in cat saliva, but in lower concentrations than Fel d 4. Urine is not a significant source of Fel d 1, but hormonal status affects its urinary levels in male cats, making it possible that litter boxes of intact male cats to be a source of this allergen at home[3,17]. Washing cats is of little benefit, because even if it reduces the amount of Fel d 1 on the skin and fur, the effect does not last long as the amount of Fel d 1 returns to its original level in just 2 d[12]. Feeding cats a diet with an egg product ingredient containing anti-Fel d 1 IgY reduces active Fel d 1 in cat saliva and dander, decreasing the environmental allergen levels[18,19].

The recombinant cat allergen rFel d 1 is produced in an *Escherichia coli* expression system by direct fusion of chain 2 and chain 1. This major allergen accounts for 60%-90% of the total allergenic activity of cat dander extracts, while specific IgE antibodies to rFel d 1 are reported in 90%-98% of European subjects with cat allergy. This is aligned with African data which revealed that nearly 75% of the patients with cat allergy from Zimbabwe have IgE antibodies against rFel d 1[12-15]. Rabbit (*Oryctolagus cuniculus*) Ory c 3 secretoglobin from saliva and dander, belonging to the same secretoglobin family, has very low sequence identity with Fel d 1, with no known IgE cross-reactivity[20]. Sequence similarity of Fel d 1 was reported with the skin brachial gland protein of an arboreal prosimian from Southeast Asia, named low loris (*Nycticebus* spp). Used for communication and defense when mixed with saliva, this gland protein has induced several cases of anaphylaxis in humans, some lethal, reported after the prosimian bites[21,22].

Fel d 1-related epithelial allergens from the majority of "big cats" (Table 2) are cross-reactive with domestic cat Fel d 1[11,23-26]. Sera from cat-allergic patients were analyzed by the first-generation solid-phase isotopic allergosorbent immunoassay using big cat fur extracts, obtained from hair collected by brushing animals (from the Natura Altis Magistra Zoo, Amsterdam, The Netherlands) at the time they were losing their winter fur. All subjects with positive skin test to cat extracts had IgE antibodies reacting with hair extracts from seven Felidae species (lion, Siberian tiger, snow leopard, jaguar, puma, ocelot, serval) but not caracal[23]. Cat-allergic individuals may be uncommonly exposed to such cross-reactive Fel d 1-related allergens in special settings, like zoos, wild parks and circus visits, but only very few cases developed severe allergic reactions upon exposure to lions and tigers in circuses[23-27]. The weight of big cats used in the past in circus entertainment is much greater than that of common domestic cats, and therefore it is likely that they produce large quantities of aeroallergens. Moreover, Siberian tiger hair extract contains 15-times more Fel d 1-like allergens *per* gram than that of lion[23].

rFel d 1 is available in singleplex and multiplex immunoassays, being considered a marker of genuine cat sensitization. It is presented together with other allergens used in singleplex and multiplex IgE assays[7,8,10,28,29] in patients with cat allergy in Table 3.

Fel d 2

The serum albumin Fel d 2 is a minor cat allergen, despite being an important protein in dander. All cats have this allergen. It is an allergen component available as a native purified and recombinant molecule in singleplex and multiplex immunoassays (Table 3). Serum albumin is a large, globular non-glycosylated protein, with α-helical structures stabilized by disulfide bridges. It is synthesized in the liver and represents a main protein constituent of plasma, with important transporter and colloid-osmotic pressure regulating roles. The amino acid identity between cat serum albumin and those of other mammals, such as dog Can f 3, pig Sus s 1, cattle Bos d 6 and horse Equ c 3, is high (75%-85% on average). Fel d 2 is considered a useful biomarker for high risk of cross-reactivity with other serum albumins[29,30-32]. Many patients allergic to cat albumin react to dog and horse albumins. About 15%-25% of cat-allergic patients are sensitized to feline serum albumin. In European allergic patients, monosensitization to Fel d 2 was found in 3.2%-7%[30-33]. There are patients with respiratory

Table 2 Cat Fel d 1 and other cross-reactive Fel d 1-related allergens from big cats (Felidae family)[11,22-25]

Subfamily	Species	Common name	Allergen
Felinae	*Felis domesticus* (*Felis catus*)	Domestic cat	Fel d 1
	Leopardus pardalis	Ocelot	Leo p 1
	Leptailurus serval	Serval	Lep s 1
	Puma concolor	Puma/cougar	Pum c 1
Pantherinae	*Panthera leo*	Lion	Pan l 1
	Panthera onca	Jaguar	Pan o 1
	Panthera pardus	Leopard	Pan p 1
	Panthera tigris longipilis	Siberian tiger	Pan t 1
	Panthera uncia (*Uncia uncia*)	Snow leopard	Unc u 1

Table 3 Allergens used in singleplex and multiplex immunoglobulin E immunoassays in patients with cat allergy[7,8,10,28,29]

Protein family	Allergen	IgE sensitization biomarker
Secretoglobins	rFel d 1	Major cat allergen, species-specific biomarker of primary sensitization to cat, as efficient as or even superior compared to natural cat extract in diagnosis
Lipocalins	rFel d 4	Major cat allergen, biomarker of cross-sensitization to other animal lipocalins, cross-reactive with lipocalins dog rCan f 6, horse rEqu q 1, and mouse nMus m 1
	rFel d 7	Minor cat allergen, biomarker of cross-sensitization to dog lipocalin, cross-reactive with lipocalin dog rCan f 1
Serum albumins	n/rFel d 2	Minor cat allergen, biomarker of sensitization to non-human serum albumin, cross-reactive with pork rSus d1/nSus s1 (cat-pork syndrome) and other serum albumins bovine nBod d 6, dog nCan f 3, and horse nEqu c 3
Immunoglobulins	nFel d 5	Minor cat allergens IgA Fel d 5 and IgM Fel d 6 carry α-Gal epitopes involved in the α-Gal syndrome and in impairing cat allergy *in vitro* diagnostics in parasite-infected patients; α-Gal biomarker: nBos d TG

Major cat allergen: Allergen recognized by immunoglobulin E antibodies of > 50% of cat allergic patients; Minor allergen: Allergen recognized by < 50% of the allergic population; IgA: Immunoglobulin A; IgM: Immunoglobulin M; α-Gal: Galactose-α-1,3-galactose; TG: Thyroglobulin, bovine.

allergy who present exclusive IgE sensitization to many serum albumins of furry animals. Regarding the clinical relevance, Fel d 2 sensitization is associated with moderate/severe rhinitis and diagnosis of asthma; it is also associated with severity of respiratory symptoms and with FeNO, as a type 2 biomarker, in young asthmatics. Moreover, high levels of IgE against Fel d 2 are associated with atopic dermatitis in children with cat allergy[34-38]. Fel d 2 is also important in relation to food allergy[1].

Cat-pork syndrome, described below[39-41], is the main food allergy phenotype in cat-allergic patients and it is secondary to the cross-reactivity of Fel d 2 with other albumins from mammals. This entity consists primarily of IgE-mediated respiratory symptoms following exposure to cats, and secondarily of food allergy symptoms after the ingestion of pork meat; therefore, the term "cat-pork syndrome" seems to be appropriate, although it is also frequently referred to as "pork-cat syndrome". The clinical picture varies from oral itching and urticaria to anaphylaxis. Fatal anaphylaxis after eating wild boar meat has also been reported. Symptoms usually occur within 30-45 min after eating pork meat, and it is not related to tick bites. Although most of the patients report reactions only to pork, some (10%-20%) report reactions to beef as well, including broiled beef intestines, but no one to cow's milk. Because albumin is a heat-labile protein, fresh meat, undercooked or dried and smoked pork are more consistent elicitors. Pork grilled meat, ribs, ham, sausages and hamburger have been mentioned as triggers. Only 1%-3% of patients who are allergic to cats seem to be at risk for allergic reactions to pork consumption, keeping in mind that only one-third of subjects who are IgE-sensitized to porcine serum albumin are likely to present food allergy to pork meat. Identification of the component-specific sensitivity pattern related to cat-pork syndrome allowed use of the cat albumin Fel d 2 and swine serum albumin nSus s 1 as markers for CRD in this clinical entity. Domestic pig (*Sus scrofa domesticus*) components nSus s 1 and rSus d 1 are available for IgE singleplex and multiplex immunoassays. These serum albumin molecules also cross-react with dog serum albumin nCan f 3 and bovine serum albumin (BSA) nBos d 6[29,42-45].

A new subphenotype of cat-pork syndrome was recently reported as anaphylaxis to BSA-containing surgical tissue adhesive (45% BSA) used as an adjunct for achieving hemostasis during cardiovascular surgery in a patient with asymptomatic long-term home exposure to cat and IgE sensitization to rFel d 1 and nFel d 2, but not to galactose-α1,3-galactose (α-Gal) containing bovine thyroglobulin. As Fel d 2 sensitization may predict cross-reactivity to nonhuman mammalian serum albumins, preoperative assessment of IgE sensitization to rFel d 2 in cat-allergic patients could be meaningful to avoid bovine and porcine surgical products[46]. BSA contained in culture media used in artificial insemination is an important anaphylaxis risk factor in patients allergic to cats, with sensitization to BSA being another possible cause of allergic reactions to some vaccines[47-49]. Moreover, equine serum albumin (also presenting high sequence identity with Fel d 2) is a causative factor of anaphylaxis to horse serum-based snake antivenom[50].

Fel d 3

Fel d 3 cystatin is a minor allergen, unavailable in commercial immunoassays. The prevalence of IgE reactivity to rFel d 3 is about 10%. It belongs to the cystatin superfamily of cysteine protease inhibitors (CPIs), being part of the stefin family. It is a small acidic protein, without cysteine residues or disulfide bonds, and having 80% sequence identity to bovine cystatin. Another animal cystatin with similar low molecular mass is Can f 8[51]. Besides Fel d 3 from cat dander, IgE-reactive cystatins have been identified in the kiwi fruit *Actinidia deliciosa* (Act d 4), *Ambrosia artemisifolia* weed pollen (Amb a CPI), and the parasitic nematode *Anisakis simplex* (Ani s 4). The sequence similarity between phytocystatin Act d 4 and other cystatins is only 13% to Fel d 3, 27% to Ani s 4, and 40% to Amb a CPI[52].

Fel d 4

The lipocalin Fel d 4 is a major allergen synthesized in cat salivary glands and found primarily in saliva in higher concentrations compared with Fel d 1. This cat allergen is involved in feline chemical communication, serving as a kairomone by eliciting defensive behavior in mice. Cat saliva is the main source of this allergen, which is deposited through grooming on the fur. Fel d 4 levels have no relation to hair length and its salivary levels appeared to be greater in neutered than intact female cats due to hormonal influences[6,8,17].

Fel d 4 is available as a recombinant molecule[10,28] in singleplex and multiplex immunoassays (Table 3). Lipocalins constitute the largest mammalian allergen family and, despite their highly conserved structure, they have variable sequence identities and cross-reactivities. The Fel d 4 cat allergen molecule has sequence identity of 67% with dog lipocalin Can f 6 and similar to horse lipocalin Equ c 1, which explains the moderate-high risk of cross-reactivity with these clinically significant allergen molecules. This is an argument for using such cross-reactive animal allergen molecules in CRD. Although Equ c 1 was regarded as a horse allergen marker, it should be considered as a highly cross-reactive molecule with the cat and dog lipocalins Fel d 4 and Can f 6. Specific IgE antibodies to Can f 6 are present in nearly 40% of patients sensitized to dogs; however, they are present in 60% of patients sensitized to both cats and dogs, which could be related to sequence identity with Fel d 4. There are patients with selective IgE reactivity to Fel d 4 but not to Equ c 1, and patients with IgE reactivity to Fel d 4 but not to Can f 6. Other major lipocalins, rabbit Ory c 4, domestic guinea pig Cav p 6, rat Rat n 1, and mouse Mus m 1, show identities between 47% and 52%. Fel d 4 shows weak cross-reactivity with the other dog lipocalin Can f 2, having less than 22% of their sequences being identical[29,53-55].

It is generally accepted that Fel d 4 lipocalin is the second most frequent sensitizing feline allergen. IgE reactivity to Fel d 4 is found in up to 63% of cat-sensitized subjects. The majority of children sensitized to Fel d 4 are also sensitized to Fel d 1 but not *vice versa*. In Central European cat-allergic patients, the sensitization rate to Fel d 4 is inferior to Fel d 1 but higher compared to Fel d 2, while monosensitization to Fel d 4 is scarce. Sensitization to this allergen molecule has relevance to the clinical presentation, as Fel d 4 is associated with the presence of asthma symptoms. Moreover, high levels of IgE to Fel d 4 are also associated with atopic dermatitis in children with cat allergy[29,33-35,56].

Fel d 5 and Fel d 6

IgA Fel d 5 and IgM Fel d 6 are present in high concentrations as Igs in cat saliva[56] and serum, and also in natural cat dander extracts, but are not used as molecular allergen components in the commercial IgE immunoassays. The IgE reactivity was found to be directed at carbohydrates of these Igs (lack of activity to deglycosylated cat

IgA) and to IgM from other animal species (rabbit, mouse, dog, pig, cow and horse) but not to human Igs. α-Gal is an IgE-binding epitope of both cat allergen Igs Fel d 5 and Fel d 6, which are cross-reactive with each other[57-59]. Serum specific IgE antibodies to the α-Gal carbohydrate epitope cause impaired *in vitro* diagnostic evaluation of cat allergy. These specific Igs may be present in patients with cat sensitization but they are not associated with rhinitis or asthma[15,29].

The glycosylated allergen component nFel d 5 present in cat dander extracts is recognized by nearly 40% of cat-sensitized European patients. Less than 20% of African patients with cat allergy have IgE against Fel d 5 compared with 66% among parasite-infected subjects without reported symptoms of cat allergy; of note, the majority (85%) of nonallergic Zimbabwean subjects with schistosomiasis and/or geohelminth infections showed anti-α-Gal IgE antibodies. The greater IgE binding to α-Gal *vs* Fel d 5 is explained by the lower number of α-Gal epitopes in nFel d 5. There is a strong correlation reported for the IgE antibody levels and cat dander extract, Fel d 5 and α-Gal specifically but not rFel d 1. The α-Gal epitope on IgA Fel d 5 is responsible for IgE anti-α-Gal reactivity to cat epithelia in parasite-infected patients[15]. Moreover, serum IgE antibodies to cat dander extract were detected among African children from rural Kenya without positive skin tests to cat epithelia extract[60], and no significant relationship was found between IgE and positive skin prick test responses to cat among South African children[61]. The α-Gal epitope is present not only on Fel d 5 and Fel d 6 but also on parasites. In addition, IgE antibodies against α-Gal are induced by tick bites. Therefore, nFel d 5 and nFel d 6 are not good markers for cat allergy diagnosis[15].

In order to decipher the problem of α-Gal cross-sensitivity in the cat IgE sensitization *in vitro* assessment, it is recommended to use at least the reliable rFel d 1 and the α-Gal biomarkers from a molecular perspective[15,29,62]. α-Gal-bearing glycoproteins are used in solid-phase immunoassays as biomarkers. Besides α-Gal coupled to human serum albumin and beef (Bos domesticus) carbonic anhydrase nBos d CA, the most widely used α-Gal markers are the recombinant human/murine chimeric monoclonal antibody cetuximab (2.04 μg α-Gal *per* mg) and the beef thyroglobulin (5.6 μg of α-Gal *per* gram). The performance characteristics in immunoassays of the last two biomarkers are relatively similar[63-67]. The bovine thyroglobulin (Bos d) α-Gal carrying molecule is commonly used in the singleplex fluorescence enzyme immunoassay with capsulated cellulose polymer as solid-phase[6,28,68]. Regarding the induction of IgE antibodies against α-Gal in humans, bites of hard ticks from the Ixodidae family are the most important primary sensitization source. The prevalence of α-Gal IgE sensitization depends on the degree of exposure to ticks[69,70]. Individuals from rural areas or with forest-related jobs have higher risk of such but only less than 10% of them present features of α-Gal syndrome[63,71-73].

The α-Gal syndrome consists of IgE-mediated allergy to α-Gal presenting as late-onset anaphylaxis after ingestion of pig, beef or lamb meat/viscera, or immediate-onset anaphylaxis to parenteral exposure to drugs containing α-Gal, such as cetuximab, snake antivenom, gelatin in plasma volume substitutes, and some vaccines[67,70]. In the α-Gal syndrome, most patients experience a decline in α-Gal-specific IgE titers by avoiding tick bites; as such, these levels should be reassessed at regular intervals[74]. The mechanisms by which parasites also induce α-Gal-specific IgE antibodies in subjects with no history of cat allergy are not elucidated but mucosal blood feeding may be involved, such as for urinary blood fluke (*Schistosoma haematobium*) or intestinal blood-feeding hookworms (*Ancylostoma duodenale*, *Necator americanus*)[15]. Keeping in mind that the human blood group B antigen represents a fucosylated α-Gal structure, some studies have revealed that individuals with blood groups AB and B may present a reduced susceptibility to IgE sensitization to α-Gal[63,73].

An association of α-Gal syndrome with anaphylaxis to pork kidney and allergic rhinoconjunctivitis with cat sensitization, presenting serum IgE to cat extract but no specific IgE to Fel d 1, has been reported[75]. Although patients allergic to red meat with specific IgE response against α-Gal are considered not to have IgE antibody responses to plant-derived cross-reactive carbohydrate determinants[67], this association is also possible[67,76]. Interestingly, α-Gal and cross-reactive carbohydrate determinants among the N-glycans of salivary glands of ticks were also reported recently[29,77].

Fel d 7

Fel d 7 is available as recombinant cat lipocalin in the singleplex fluorescence enzyme immunoassay with capsulated cellulose polymer solid-phase and the new generation

macroarray nanotechnology-based multiplex immunoassay[10,28] (Table 3). It was reported to bind IgE in approximately 40% of subjects with rhinoconjunctivitis and/or asthma exposed to cats. Almost 20% of patients with Fel d 7-specific IgE do not have detectable IgE against Fel d 1. Fel d 7 is present in small quantities in natural extracts. The concentration of this lipocalin in cat hair extracts is approximately 0.24 μg/mL. Fel d 7 is a von Ebner gland protein isolated from the posterior region of the cat tongue, known to contain lingual salivary glands. It shares a high sequence identity (62%) with the major dog allergen Can f 1, giving it high potential for cross-reactivity with Can f 1. Thus, Fel d 7 may contribute to respiratory allergy symptoms not only in cat but also in dog-allergic patients. Because the concentration of Fel d 7 in cat saliva is about 4 mg/mL, it is plausible that cat licking may be a route for the sensitization to Fel d 7 along with the inhalation of aerosolized allergen[29,78-81].

Fel d 8

Fel d 8 is a distinct latherin-like protein. The frequency of IgE binding of sera from patients with respiratory cat allergy to rFel d 8 is nearly 20%. The IgE binding to Fel d 8 is highly correlated with binding to Fel d 1. Fel d 8 is not usually detected in natural cat dander extracts, being found in the saliva of cats and isolated from their submandibular salivary gland[78]. It has a high degree of homology to horse Equ c 4 and Equ c 5. Equ c 5 is an allergen that binds IgE in 77% of horse-allergic patients, and rEqu c 4 is available in the new macroarray multiplex immunoassay[10,80,81]. Fel d 8 belongs to the lipopolysaccharide-binding protein/bactericidal permeability-increasing family[81] and it is not yet available in the commercial IgE immunoassays.

Other cat allergens

Fel d S100, a calcium-binding protein detected in cat saliva, and Fel d Hp, a haptoglobin detected in blood, are two additional allergens mentioned in the Allergome database[11,81], also not currently available in commercial immunoassays. S100A12 and haptoglobin are undenominated IgE binding proteins. The IgE antibody response to S100A12 is of low prevalence, but the specific IgE titer could be high in some individuals. This is of interest as it suggests inhalation of this calgranulin inflammatory mediator, known to have interspecies activity. IgE binding to plasma haptoglobin is infrequent, but significantly more IgE binding was found in subjects with cat-allergy than in those without allergy. The likely source of exposure to this acute phase protein is saliva from cats with poor gingival hygiene[81].

Because a frequent association between cat and dog sensitization is known for several decades, and a common question is whether this is due to co-sensitization to different allergen components or cross-reactivity between cat and dog allergenic molecules, a short presentation of additional allergens related to this aspect is needed.

Cross-reactivity between cat and dog allergens is usually explained by high-sequence homologies or structural similarities between lipocalins Fel d 4 and Can f 6, albumins Fel d 2 and Can f 3, as mentioned above, but recently a cat Niemann-Pick type C2 (Cat-NPC2) allergenic protein, a homologue of Can f 7, was also detected in cat dander extracts. Can f 7 shares 78% sequence identity with Cat-NPC2, and this clearly indicates the possible cross-reactivity between them. rCat-NPC2 can bind specific IgE in at least 14.5% of cat-allergic subjects[82]. This newly identified and characterized animal allergen has the potential of becoming a useful tool for CRD, but it is not yet available in commercial IgE immunoassays. Interestingly, cross-reactivity was observed also between Cat-NPC2 and Der f 2 (also belonging to the NPC2 family of proteins) indicating a possible association between IgE sensitizations to cat, dog and house dust mites[82].

Moreover, a previous report demonstrated the presence of a Fel d 1-like allergen with a molecular weight of 20 kDa in dog dander extracts, which may be responsible for *in vitro* double positivity to cat and dog. The clinical significance of this cross-reactivity is not clear since no patients with IgE cross-reactivity to this Can f CRA (Fel d 1 cross-reactive allergen) revealed clinical symptoms to dogs[83].

Regarding kallikrein allergens, no patterns of cross-reactivity of cat allergens with male dog prostatic kallikrein Can f 5 have been identified to date. Therefore, even if there are few case reports of human seminal plasma allergy in women sensitized to Can f 5 from dog urine and dander[29,84-86], no such cross-reactivity reactions have been published in cat allergic patients.

MOLECULAR APPROACH TO CAT ALLERGY

The molecular approach to cat allergy involves allergen components used in singleplex and multiplex immunoassays for *in vitro* diagnosis, presented in Table 3. The designation of allergen names is derived from the source, the first three letters of the genus, the first letter of the species, and a number indicating the chronology of the discovery, for example, Fel d 1 is the first allergen from the domestic cat *Felis domesticus*[87]. The common exposure to these allergen molecules includes different indoor settings, such as homes with cats as pets, but also in schools, daycare centers, public buildings, workplaces, and public transport vehicles, particularly if pet ownership is more prevalent in the area[88] because of their transportability on clothing[89]. A popular misconception persists regarding cat allergy related to the belief that certain cat breeds produce less allergens and are 'hypoallergenic' due to their fur type[90]. The major allergen Fel d 1 is produced by the cat's sebaceous glands, and, together with Fel d 4, is detected in the saliva and distributed on the fur by grooming. In common neutered domestic cats, fur length and color or body size did not relate Fel d 1 levels in reservoir dust from homes. Fel d 4 levels are also not related to hair length, however, neutered female cats have higher levels compared to unneutered ones[17,86,91]. There have been attempts to obtain so-called 'allergy-free' transgenic cats characterized by the absence of Fel d 1, by disrupting the coding sequence of the target gene with a specialized construct[92] or by CRISPR-Cas9-mediated genomic editing of Fel d 1[93]. To date, there are no hypoallergenic or allergen-free cats[1].

The diagnosis of cat allergy may seem uncomplicated at first glance, since most patients react to the main allergen molecule Fel d 1, but it is important to keep in mind that the natural cat dander extracts used for diagnosis, while containing this allergen mainly, differ in the quality and quantity of cat individual allergens and other molecules. Moreover, contamination of commercially available animal dander extracts with house dust mite allergens is possible and may induce *in vivo* false-positive responses. CRD using individual allergenic proteins can improve the diagnosis of mammalian pet allergy[56,94-96].

Recombinant and well-defined allergen components have great advantages for CRD immunoassays used to assess IgE sensitization patterns to cat allergen components; these include primary sensitization and presence of allergy, polysensitization and presence of severe allergy, secondary sensitization, cross-reactivity to other furry animals, and irrelevant sensitization[1,86]. In patients suspected of cat allergy, Fel d 1, Fel d 2 and Fel d 4 seem to be the most important allergen components to assess. IgE sensitization to more than three cat allergen molecules in children is superior in predicting future cat symptoms than sensitization to cat extract, and sensitization to the major species-specific allergen is a predictor of cat allergy at adult age[9,29]. Sensitization to Fel d 1 is associated with asthma, and polysensitization (Fel d 1, Fel d 2 and Fel d 4) is associated with both clinical reactivity to cat and also bronchial responsiveness and increased FeNO as a type 2 inflammation biomarker. Asthmatic children with cat allergy have higher Fel d 1-specific IgE levels than children with rhinitis only. Asthma symptoms to cat exposure are associated with specific IgE antibodies to cat allergens Fel d 1 and Fel d 4 in cat-allergic children. Moreover, IgE sensitization to Fel d 2 and Fel d 4 is associated with atopic dermatitis in children with cat allergy[86].

CONCLUSION

The benefits of molecular diagnosis in cat allergy involve the use of the cat-specific major allergen as a species-specific molecule, cross-reacting allergen components, including those present in small quantities in natural extracts, while considering those impairing the *in vitro* allergy diagnosis. Identification and characterization of molecular cat allergens allowed their use in singleplex and multiplex immunoassays for a precision diagnostic approach, with assessing their clinical significance and the association with cat allergy phenotypes and severity[29].

The manifestations of cat allergy vary widely, from rhinitis and conjunctivitis to severe asthma. Other than respiratory and ocular allergy, cat licks can cause contact urticaria upon exposure to the saliva, while cat bites can cause anaphylaxis in patients sensitized to cats[2,97,98]. IgE sensitization to cat epithelia increases the risk of patients to develop asthma or rhinitis. In addition, persistent atopic dermatitis lesions occur more often in patients sensitized to cat dander. There is also clear evidence for the clinical importance of assessing cat allergen components in relation to both α-Gal and

cat-pork syndrome[29].

Allergenic molecules induce specific IgE sensitization of mast cells and trigger type 2 allergic inflammation upon re-exposure. The availability of natural purified or recombinant allergens improved the understanding of the molecular mechanisms leading to these immune responses, which vary depending on several structural and biological characteristics of these allergens. In addition, other pro-inflammatory properties of some allergens must be mentioned, including late-phase allergic inflammation induced by non-IgE reactive peptides of Fel d 1 *via* major histocompatibility complex-restricted T cell activation[99-101].

The molecular approach for cat allergy allows a better understanding of the exposure and immune response to feline allergens, the relationship of these specific IgE responses to symptoms, and their clinical relevance[29].

Identification of cat allergen-specific IgE antibodies, either bound to mast cells by skin prick tests or in serum by immunoassays, detects IgE sensitization, a condition necessary but not sufficient to make the definitive diagnosis of cat allergy[100]. CRD, with or without *in vivo* tests, must be used within the framework of a detailed clinical history, because IgE sensitization does not necessarily imply clinically relevant allergy[86,99,100]. A deeper *in vitro* analysis with the help of IgE immunoassays using molecular allergens creates the bigger picture of the patient IgE sensitization profile in order to assess genuine sensitization, primary sensitization source, co-sensitization, cross-reactivity and allergy risks, including prediction of allergy severity[1,86].

Precision allergy molecular diagnostic applications (PAMD@) in cat allergy involve several molecular allergens used in commercial singleplex and multiplex IgE immunoassays, Fel d 1, Fel d 2, Fel d 4 and Fel d 7, these being the allergenic components currently available on the market[100]. For other native or recombinant allergenic components to be included in such immunoassays used in clinical practice, they must not only be well characterized and experimentally validated, but must also be clinically validated and available from their production point of view. Moreover, the characteristics of the solid-phase of the immunoassay and the manner by which allergenic molecules are coupled are important to reflect their biochemical properties and specific requirements for stability, preserving epitope complexity. Regarding native IgA Fel d 5 and IgM Fel d 6 allergen components with α-Gal IgE-binding epitopes, their use may be associated with analytical errors and impaired *in vitro* diagnostics in some patients, in such cases bovine thyroglobulin being a good molecular biomarker for α-Gal IgE sensitization[5,15,28,29,86]. Although α-Gal is present on cat Igs, cross-sensitization between cat allergens and the oligosaccharide antigen is not considered clinically relevant[100].

Concerning cat allergen immunotherapy, although some patients may likely benefit more from it, particularly those with moderate-to-severe disease, monosensitized to Fel d 1[102], and a good immune and clinical response to subcutaneous immunotherapy is associated with high doses of major allergens in the cat allergen extracts[103], more data are required from large trials to obtain more definitive conclusions. Summing-up, cat allergy CRD, recently proposed to be termed as PAMD@ by the updated World Allergy Organization consensus document[100], allows for an accurate and detailed assessment of patients' IgE sensitization profiles and may facilitate individualized management options[88,100].

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MINIREVIEWS

Concise review of stereotactic irradiation for pediatric glial neoplasms: Current concepts and future directions

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Abstract

Brain tumors, which are among the most common solid tumors in childhood, remain a leading cause of cancer-related mortality in pediatric population. Gliomas, which may be broadly categorized as low grade glioma and high grade glioma, account for the majority of brain tumors in children. Expectant management, surgery, radiation therapy (RT), chemotherapy, targeted therapy or combinations of these modalities may be used for management of pediatric gliomas. Several patient, tumor and treatment-related characteristics including age, lesion size, grade, location, phenotypic and genotypic features, symptomatology, predicted outcomes and toxicity profile of available therapeutic options should be considered in decision making for optimal treatment. Management of pediatric gliomas poses a formidable challenge to the physicians due to concerns about treatment induced toxicity. Adverse effects of therapy may include neurological deficits, hemiparesis, dysphagia, ataxia, spasticity, endocrine sequelae, neurocognitive and communication impairment, deterioration in quality of life, adverse socioeconomic consequences, and secondary cancers. Nevertheless, improved understanding of molecular pathology and technological advancements may pave the way for progress in management of pediatric glial neoplasms. Multidisciplinary management with close collaboration of disciplines including pediatric oncology, surgery, and radiation oncology is warranted to achieve optimal therapeutic outcomes. In the context of RT, stereotactic irradiation is a viable treatment modality for several central nervous system disorders and brain tumors. Considering the importance of minimizing adverse effects of irradiation, radiosurgery has attracted great attention for clinical applications in both adults and children. Radiosurgical applications offer great potential for improving the toxicity profile of radiation delivery by focused and precise targeting of well-defined tumors under stereotactic immobilization and image guidance. Herein, we provide a concise review of stereotactic irradiation for

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pediatric glial neoplasms in light of the literature.

Key Words: Radiosurgery; Stereotactic irradiation; Stereotactic radiosurgery; Pediatric glioma; Gamma knife; Linear accelerator

Core Tip: Pediatric gliomas comprise the majority of brain tumors in children. Radiotherapeutic management of pediatric gliomas poses a formidable challenge considering the adverse effects of irradiation for this vulnerable patient population. In this context, efforts have been focused on improving the toxicity profile of radiation delivery. Stereotactic irradiation with stereotactic radiosurgery or stereotactic radiotherapy in a single or few treatment fractions may serve as a viable radiotherapeutic approach to achieve this goal given the high conformality along with steep dose gradients around the target volume allowing for reduced normal tissue exposure under precise immobilization and image guidance.

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INTRODUCTION

Brain tumors, which are among the most common solid tumors in childhood, remain a leading cause of cancer-related mortality in pediatric population[1-3]. Gliomas, which may be broadly categorized as low grade glioma (LGG) and high grade glioma (HGG), account for the majority of brain tumors in children[4]. Expectant management, surgery, radiation therapy (RT), chemotherapy, targeted therapy or combinations of these modalities can be used to manage pediatric gliomas. Several patient, tumor and treatment-related characteristics including age, lesion size, grade, location, phenotypic and genotypic features, symptomatology, predicted outcomes and toxicity profile of available therapeutic options should be considered in decision making for optimal treatment[4-6]. Management of pediatric gliomas poses a formidable challenge to the physicians owing to concerns about treatment induced toxicity. Adverse effects of therapy for this vulnerable patient population may include neurological deficits, hemiparesis, dysphagia, ataxia, spasticity, endocrine sequelae, growth abnormalities, audiovisual toxicity, neurocognitive and communication impairment, deterioration in quality of life, adverse socioeconomic consequences, and secondary cancers[7-10]. Nevertheless, improved understanding of molecular pathology and technological advancements may improve management of pediatric glial neoplasms. Multidisciplinary management with close collaboration of disciplines including pediatric oncology, surgery, and radiation oncology is warranted to achieve optimal therapeutic outcomes[11-14].

In the context of RT, stereotactic irradiation represents a viable treatment modality for several central nervous system disorders (CNS) and brain tumors[15-19]. Considering the importance of minimizing adverse effects of irradiation, radiosurgery has attracted critical attention for clinical applications in both adults and children. Radiosurgical applications offer great potential for improving the toxicity profile of radiation delivery by focused and precise targeting of well-defined tumors under stereotactic immobilization and image guidance. Herein, we provide a concise review of ste-reotactic irradiation for pediatric glial neoplasms in light of the literature.

STEREOTACTIC IRRADIATION FOR PEDIATRIC HGG

Based on the classification of World Health Organization (WHO) in 2016, HGG comprises glioblastoma, anaplastic astrocytoma, and diffuse midline glioma including

diffuse intrinsic pontine glioma (DIPG)[20]. Pediatric HGG accounts for approximately 8%-12% of all childhood CNS tumors and it is the leading cause of cancer-related mortality in children under 19 years of age[21-24]. Pediatric HGG usually follows an aggressive disease course which results in morbidity and mortality, however, there are several distinctive features of pediatric HGG regarding natural history, causative genetic mutations, response to treatment, and tumor localization within the brain[6,22,25-28]. While HGG frequently arises from LGG with malignant transformation in adults, this is very uncommon in pediatric patients with differences in genetic and epigenetic features. Similar to adult HGG, surgery is the primary treatment modality for management of pediatric HGG, and the extent of resection is a significant prognostic factor[29-34]. Surgery alone may be insufficient for optimal management, and adjunctive therapies including RT and chemotherapy are recommended. Gross total resection of HGG is usually difficult owing to the infiltrative nature of the disease and the risk of excessive toxicity particularly when the lesions are located in close vicinity of critical neurovascular structures[22,35,36]. Microscopic tumor cells may still remain even after gross total resection with potential for subsequent recurrence. Due to the increased vulnerability of younger children to adverse effects of ionizing radiation and the relatively favorable disease course, RT is typically deferred for this subgroup of patients under 3 years of age by considering other therapeutic options[37-39]. Never-theless, older children are frequently referred for postoperative RT with concurrent and adjuvant chemotherapy[6,22,29,40]. In the context of RT for pediatric HGG, con-ventional fractionation is common practice owing to lack of superiority of altered fractionation regimens[41-44]. Of note, several series investigated the utility of hypo-fractionated RT regimens especially for DIPG[44-47]. Compared to conventionally fractionated RT delivered over 5 wk to 6 wk, hypofractionated RT schedules may offer reduction in number of anesthesia administrations for patients treated under anesthesia and less burden on patients, parents, and treatment centers.

Radiation dose escalation strategies, combined modality treatment approaches, and incorporation of contemporary RT techniques such as radiosurgery are being investigated to improve the therapeutic ratio for HGG in view of the aggressive disease course and poor treatment outcomes despite intensive management. Stereotactic irradiation is a common RT technique for treatment of adult HGG and several studies support its use for this indication either as part of initial management or as salvage therapy[18,19,48-51]. Data on stereotactic irradiation of HGG have been mostly extracted from the literature including adult patients considering that there is paucity of data about pediatric HGG. Survival after hypofractionation (including radiosurgical treatments) in glioblastoma has been assessed in a recent meta-analysis and systematic review[52]. Meta-analysis of eleven comparative studies regarding first line management of glioblastoma with hypofractionated *vs* conventionally fractionated irradiation revealed no significant difference between the two fractionation schemes, and hypofractionation has been suggested as a reasonable alternative fractionation scheme for selected patients[52]. In the context of radiosurgery, a phase III randomized trial conducted by the Radiation Therapy Oncology Group reported no survival advantage with the addition of stereotactic irradiation to conventional external beam RT[53]. Nevertheless, there is active investigation on the utility of stereotactic irradiation for achieving improvements in treatment outcomes of patients with HGG. Stereotactic irradiation is an extreme form of focal RT which is used to deliver high doses of radiation in a single or a few fractions to well-defined lesions. Minimal exposure of normal tissues due to steep dose gradients around the target volume may be achieved with radiosurgery. While several studies have reported improved treatment outcomes with incorporation of stereotactic irradiation for adult HGG, there is paucity of data on the utility of radiosurgery for pediatric HGG[54-61].

Giller *et al*[58] reported outcomes of robotically guided radiosurgery for pediatric brain tumors. Twenty-one patients aged between 8 mo and 16 years received Cyberknife radiosurgery for pilocytic astrocytomas, anaplastic astrocytomas, ependymomas, atypical teratoid/rhabdoid tumors, medulloblastomas, cranio-pharyngiomas, and other pathologies which were deemed unresectable[58]. Local control was achieved in patients with anaplastic astrocytoma, and the authors concluded that Cyberknife radiosurgery could be used for achieving local control of selected pediatric brain tumors with elimination of the requirement for rigid head fixation[58]. In another series of 90 children receiving stereotactic radiosurgery (SRS) for brain tumors at the Joint Center for Radiation Therapy during a 10-year period between 1987 and 1997, 20% of the patients (18 patients) had pediatric HGG[59]. Out of the total 90 patients, 10 patients (11%) had glioblastoma and 8 patients (9%) had anaplastic astrocytoma[59]. Median progression free survival (PFS) was 12 mo (range: 3-119 mo)

and median 3-year actuarial local control rate was 50% for the 18 patients with glioblastoma and anaplastic astrocytoma[59]. Four patients receiving SRS as part of initial management were alive and free of progression at 50, 62, 66, and 119 mo, respectively[59]. Baumann *et al*[60] reported their experience with pediatric radiosurgery in a series of 52 patients. Local control was worse in patients with HGG compared to LGG[60]. Grabb *et al*[61] assessed the role of SRS in 25 pediatric patients with surgically incurable glial tumors treated between 1988 and 1994. Twelve patients had malignant astrocytomas or ependymomas. While 7 children died of disease with a median survival of 6 mo after SRS, 5 children were alive at 12, 45, 50, 72, and 72 mo after radiosurgical management[61].

In summary, stereotactic irradiation may be considered as a viable therapeutic strategy for management of adult HGG particularly in the recurrent disease setting. There is scarce literature regarding the utility of stereotactic irradiation for HGG in children, however, this advanced radiotherapeutic technology may offer benefits for pediatric patients and deserves further investigation to improve normal tissue sparing through precise stereotactic localization under image guidance.

STEREOTACTIC IRRADIATION FOR PEDIATRIC LGG

Pediatric LGG is the most common CNS neoplasm among children[5,30]. Most common subtype of pediatric LGG is pilocytic astrocytoma, and other subtypes are diffuse astrocytoma (fibrillary, gemistocytic, or protoplasmic), subependymal giant cell astrocytoma, pilomyxoid astrocytoma, pleomorphic xanthoastrocytoma, gangliglioma, gangliocytoma, desmoplastic infantile ganglioglioma, and dysembryoplastic neuroepithelial tumor[5,23]. Prognosis for these heterogeneous group of tumors is usually favorable, thus toxicity profile of management is very important[62-65]. Location and extent of disease are critical factors which should be considered in decision making for treatment of pediatric LGG. Other important factors include age, symptomatology, phenotypic and genotypic features, predicted outcomes and toxicity profile of available therapeutic options. Optimal care of patients with pediatric LGG may require incorporation of multimodality management with close collaboration of pediatric oncology, surgery, and radiation oncology disciplines[64,65]. Surgical resection is the principal mode of management for tumors which are amenable to surgery. Observation may be considered after surgical removal of the tumor to spare pediatric patients from potential toxicity of adjunctive therapies. Previous data on pediatric and adult patients have shown improvements in treatment outcomes with incorporation of RT in management of LGG[66,67]. There have been significant advances in the disciplines of pediatric neurosurgery and radiation oncology over the years[12-14]. Despite advances in therapy, irradiation for pediatric brain tumors still remains to be a challenge given the vulnerability of children to adverse RT effects such as neuroendocrine and neurocognitive deficits, growth abnormalities, audiovisual toxicity, deterioration in quality of life, adverse socioeconomic consequences, and secondary cancers[7-10,68]. Nevertheless, optimal surgical management may not be feasible for tumors at critical locations such as the optic pathway, brainstem, basal ganglia, thalamus, hypo-thalamus, and other eloquent brain areas. Therefore, irradiation in the form of radiosurgery or conventionally fractionated RT may be considered in the presence of surgically inaccessible tumors, incomplete excision, or recurrence. Conformal RT techniques, particle therapy, and radiosurgical treatments may offer reduced normal tissue exposure in management of pediatric LGG[68-72]. Among the radiotherapeutic options for treatment of pediatric LGG, stereotactic irradiation offers a viable RT technique. Radiosurgery is a very highly focused form of therapeutic irradiation with the potential of improving the toxicity profile of radiation delivery through steep dose gradients around the target volume. Pilocytic astrocytomas, the most common of pediatric LGG, are typically visualized as well-defined lesions on neuroimaging which renders them more suitable for radiosurgical management. While infiltrative nature of the disease comprises a challenging aspect in radiosurgery for HGG, most LGG lesions with well-defined borders are suitable for treatment with stereotactic irradiation. Several studies including pediatric patients have addressed the utility of stereotactic irradiation in LGG management either as primary, adjuvant, boost or salvage therapy[73-88]. Table 1 shows summarized data from selected series of stereotactic irradiation for LGG including pediatric patients.

Barcia *et al*[73] reported their experience with SRS for deeply seated inoperable LGG in a series of 16 patients including 8 children. Histological confirmation of LGG was available for 7 patients, and 12 patients had received prior irradiation. Median age was

Table 1 Selected series of stereotactic irradiation for low grade glioma including pediatric patients

Ref.	Study period	Number of patients	Proportion of pediatric patients (%)	Histology	Setting	Treatment	Dose (Gy)	Age (yr)	Tumor size	Prior irradiation	Follow-up duration	Tumor control or PFS (%)
Barcia *et al*[73], 1994	1978-1991	16	50	LGG	Primary or boost therapy	SRS by use of a cobalt source and stereoguide	Mean margin dose 21.7 Gy	Median age 20 yr (range: 4-68 yr)	-	12 patients	Median 50 mo	Tumor control 81
Somaza *et al*[74], 1996	1990-1993	9	100	Pilocytic astrocytoma	Adjuvant or salvage therapy	GKSRS	Median margin dose 15 Gy	Mean age 8.6 yr (range: 4-17 yr)	Mean tumor diameter 16 mm	2 patients	Median 19 mo	Tumor control 100
Kida *et al*[75], 2000	2000	12 (total number of patients in the study is 51)	100	WHO Grade I low grade astrocytoma	As part of initial management or salvage therapy	GKSRS	Mean margin dose 12.5 Gy	Mean age 9.8 yr	Mean tumor diameter 25.4 mm	-	Mean 27.6 mo	Tumor control 91.7
Boëthius *et al*[76], 2002	1978-1997	19	84.2	Pilocytic astrocytoma	Adjuvant therapy	GKSRS	Median margin dose 10 Gy	Mean age 10.6 yr (range: 2-60 yr)	Median 2.2 cc	2 patients	Median radiological follow-up 4.7 yr	Tumor control 94.7
Hadjipanayis *et al*[77], 2003	1987-2000	49	59	Pilocytic astrocytoma (37 patients) and WHO Grade II fibrillary astrocytoma (12 patients)	As part of initial management or salvage therapy	GKSRS	Median margin dose 15 Gy	Median age 14 yr (range: 3-52 yr) for patients with pilocytic astrocytoma and median age 25 yr (range: 5-57 yr) for patients with WHO Grade II fibrillary astrocytoma	Median 3.3 cc	13 patients	Median 32 mo after SRS	Tumor control 67
Saran *et al*[78], 2002	1994-1999	14	100	LGG	As part of initial management or salvage therapy	LINAC-based SRT	Total dose 50-55 Gy	Median age 8 yr (range: 5-16 yr)	Median 19.5 cc	0 patient	Median 33 mo	PFS 87 at 3 yr
Marcus *et al*[79], 2005	1992-1998	50	-	WHO Grade I-II astrocytoma	Salvage therapy	LINAC-based SRT	Mean total dose 52.2 Gy	Median age 9 yr (range: 2-26 yr)	≤ 5 cm in maximal dimension in all patients	0 patient	Median 6.9 yr	PFS 82.5 at 5 yr, PFS 65 at 8 yr
Wang *et al*[80], 2006	1993-2003	21	-	LGG	Primary, boost, adjuvant or salvage therapy	GKSRS	Median margin dose 14.5 Gy	Median age 20 yr (range: 6-70 yr)	Median 2.4 cc	7 patients	Median radiological follow-up 49 mo	Tumor control 67
Kano *et al*[81], 2009	1987-2006	50	100	Pilocytic astrocytoma	As part of initial management or salvage therapy	GKSRS	Median margin dose 14.5 Gy	Median age 10.5 yr (range: 4.2-17.9 yr)	Median 2.1 cc	5 patients	Median 55.5 mo	PFS 70.8 at 5 yr

Henderson *et al*[82], 2009	1997-2004	12	-	WHO Grade I LGG (10 patients), WHO Grade II LGG (2 patients)	As part of initial management or salvage therapy	GKSRS	Median margin dose 13 Gy	Median age 17.4 yr (range: 5.9-63 yr)	Median 4.4 cc	4 patients	Median 48.2 mo	PFS 75 at 4 yr
Weintraub *et al*[83], 2012	1989-2011	24	100	LGG	As part of initial management or salvage therapy	GKSRS	Median margin dose 15 Gy	Median age 11 yr (range: 4-18 yr)	Mean 2.4 cc	3 patients	Median imaging follow-up 74 mo	Tumor control 83
Hallemeier *et al*[84], 2012	1992-2005	18	33	Pilocytic astrocytoma	As part of initial management or salvage therapy	GKSRS	Median margin dose 15 Gy	Median age 23 yr (range: 4-56 yr)	Median 9.1 cc	10 patients	Median 8 yr	PFS 41 at 5 yr
Lizarraga *et al*[85], 2012	1995-2010	12	41.7	Pilocytic astrocytoma	Salvage therapy	LINAC-based SRS or SRT	Median dose 18.75 Gy for SRS and median dose 50.4 Gy for SRT	Median age 21 yr (range: 5-41 yr)	Median 6.5 cc for SRT and median 1.69 cc for SRS	0 patient	Median 37.5 mo	PFS 73.3 at long term
Simonova *et al*[86], 2016	1992-2002	25	100	Pilocytic astrocytoma	As part of initial management or salvage therapy	GK-based SRS or SRT	Median margin dose 16 Gy for patients receiving single fraction, median dose 25 Gy for SRT	Median age 13 yr (range: 3-17 yr)	Median 2.7 cc	2 patients	Median 15 yr	PFS 80 at 10 yr
Trifiletti *et al*[87], 2017	1990-2015	28	-	Pilocytic astrocytoma	As part of initial management or salvage therapy	GK-based SRS or SRT	Median margin dose 16 Gy	Median age 17.4 yr (range: 2-70.3 yr)	Median 1.84 cc	4 patients	Median 5.4 yr	PFS 96 at 6 yr
Gagliardi *et al*[88], 2017	2001-2014	39	23.8	LGG	As part of initial management or salvage therapy	GKSRS	Median margin dose 15 Gy	Median age 31 yr (range: 9-72 yr)	Median 1.24 cc	8 patients	Median 54.5 mo	PFS 52.8 at 5 yr

LGG: Low grade glioma; SRS: Stereotactic radiosurgery; GKSRS: Gamma knife stereotactic radiosurgery; WHO: World Health Organization; PFS: Progression free survival; LINAC: Linear accelerator; SRT: Stereotactic radiotherapy.

20 years (range: 4-68 years). Cobalt source and stereo guide were used for either primary or boost therapy with a mean margin dose of 21.7 Gy. Complete response was achieved for 8 patients (50%), and tumor shrinkage or stabilization was detected in 5 patients (31%) corresponding to a tumor control rate of 81%. Three patients (19%) who had brainstem glioma succumbed to their disease with no response to SRS. The authors concluded that radiosurgery could serve as an effective therapeutic modality for management of deeply seated LGG[73].

Somaza *et al*[74] from Pittsburgh University investigated the role of gamma knife SRS (GKSRS) in adjuvant treatment of 9 children with deeply seated, growing and unresectable pilocytic astrocytomas. Lesions had a mean diameter of 16 mm and were localized at cerebellar peduncle, dorsolateral pons, midbrain, thalamus, hypothalamus, caudate nucleus, and temporal lobe. Mean margin dose was 15 Gy. At a mean follow-up duration of 19 mo, tumor control was achieved in all patients with

significant tumor shrinkage in 5 patients and no further growth in 4 patients. No patients suffered from early or late toxicity. The authors concluded that GKSRS proved to be safe and effective for management of deeply seated and small volume pilocytic astrocytomas[74].

Kida *et al*[75] reported long term outcomes of GKSRS in the management of low grade astrocytomas in a large series of 51 patients from Japan. The study included 12 pediatric patients with a mean age of 9.8 years. Tumor control rate was 91.7% for WHO grade I astrocytomas and 87.2% for WHO grade II astrocytomas. Mean margin dose was 12.5 Gy for WHO grade I astrocytomas and 15.7 Gy for WHO grade II astrocytomas. Higher treatment response was achieved in patients ≥ 10 years of age with WHO grade I astrocytomas and for those with follow-up duration exceeding 2 years. The authors concluded that radiosurgery could play an important role in management of low grade astrocytomas and complete cure could be expected at least for some patients[75].

Boëthius *et al*[76] from Sweden reported outcomes of 19 patients receiving GKSRS for pilocytic astrocytoma at Karolinska Hospital. Mean age was 10.6 years (range: 2-60 years) and the study group included 16 pediatric patients. Median tumor volume was 2.2 cc. A median marginal dose of 10 Gy was used since majority of tumors were located within or in close vicinity of the brainstem. At a median radiological follow-up duration of 4.7 years and median clinical follow-up duration of 7 years, a satisfactory tumor control rate of 94.7% was achieved despite the relatively lower GKSRS dose[76].

Hadjipanayis *et al*[77] assessed outcomes of 49 patients (including 29 children) receiving GKSRS at the Pittsburgh University for LGG. Involved locations included the brainstem in 22 patients, thalamus in 6 patients, temporal lobe in 5 patients, cerebellum in 4 patients, frontal lobe in 4 patients, parietal lobe in 3 patients, insular cortex in 1 patient, hypothalamus in 1 patient, third ventricle in 1 patient, corpus callosum in 1 patient, and optic tract in 1 patient. Median age was 14 years (range: 3-52 years) for the 37 patients with pilocytic astrocytoma including 25 children aged ≤ 18 years. Median age was 25 years (range: 5-57 years) for the 12 patients with WHO Grade II fibrillary astrocytoma including 4 children aged ≤ 18 years. Median margin dose was 15 Gy and 16 Gy for pilocytic astrocytomas and WHO Grade II fibrillary astrocytomas, respectively. Overall, serial neuroimaging after GKSRS revealed complete tumor resolution in 11 patients, reduced tumor volume in 12 patients, stable tumor volume in 10 patients, and delayed tumor progression in 16 patients. Out of the 37 patients with pilocytic astrocytoma, tumor control was achieved in 25 patients (68%). Out of the 12 patients with WHO Grade II fibrillary astrocytoma, tumor control was achieved in 8 patients (67%). The authors concluded that SRS offers a safe and promising therapeutic modality for management of selected patients with pilocytic astrocytomas or WHO Grade II fibrillary astrocytomas[77].

Saran *et al*[78] from Royal Marsden Hospital reported outcomes of stereotactically guided conformal radiotherapy (SCRT) in the management of progressive or inoperable pediatric LGG. Median age was 6 years (range: 5-16 years). Fourteen patients received linear accelerator (LINAC)-based SCRT in 30-33 daily fractions, and the total dose was 50-55 Gy. Lesion locations included the optic chiasm in 9 patients, third ventricle in 2 patients, pineal region in 1 patient, craniocervical junction in 1 patient, and hypothalamus in 1 patient. Median tumor volume was 19.5 cc (range: 7.5-180 cc). Median follow-up duration was 33 mo. The 3-year local PFS and overall survival rate following SCRT was 87% and 100%, respectively. The authors concluded that SCRT offers a feasible and high precision technique for stereotactic irradiation of pediatric LGG[78].

Marcus *et al*[79] from Dana-Farber Cancer Institute assessed the efficacy of LINAC-based stereotactic radiotherapy (SRT) for management of small, localized, pediatric brain tumors. Their prospective study included 50 patients with LGG. Out of the 50 patients, 35 patients had WHO grade I astrocytoma and 15 patients had WHO grade II astrocytoma. Median age was 9 years (range: 2-26 years). Out of the 50 patients, 38 patients had progression after surgery and 12 patients had progression after chemotherapy. Mean total dose for SRT was 52.2 Gy delivered in 1.8-Gy daily fractions. With a median follow-up duration of 6.9 years, PFS rate was 82.5% at 5 years and 65% at 8 years. Overall survival was 97.8% and 82% at 5 and 8 years, respectively. There were 6 cases of local progression all within the primary tumor bed. There was no marginal failure. The authors concluded that SRT offers excellent local control for small, localized LGG in children and limited margins with stereotactic immobilization and planning techniques could be considered to minimize late sequelae in view of no marginal failures in the study[79].

Wang *et al*[80] reported outcomes of GKSRS for 21 patients with 25 histologically proven low grade astrocytomas treated at the Taipei Veterans General Hospital.

Median age was 20 years (range: 6-70 years). Median margin dose was 14.5 Gy. With a median radiological follow-up duration of 49 mo and median clinical follow-up duration of 67 mo, all patients with pilocytic astrocytoma were free from tumor progression. Complete tumor remission was achieved in 3 patients. PFS rate was 65% at 10 years. The authors suggested reduction in GKSRS dose to prevent excessive toxicity in the setting of combined use of GKSRS and RT. The authors concluded that GKSRS may be utilized for management of selected patients with low grade astrocytomas to achieve durable long term local tumor control rates with acceptable toxicity[80].

Kano *et al*[81] from Pittsburgh University assessed GKSRS outcomes for management of newly diagnosed or recurrent juvenile pilocytic astrocytomas. Their series included 50 pediatric patients with a median age of 10.5 years (range: 4.2-17.9 years). Lesion locations included the cerebellum in 20 patients, brainstem in 13 patients, cerebral hemispheres in 7 patients, basal ganglia in 6 patients, and ventricles in 4 patients. Out of the total 50 patients, only 5 patients had received prior fractionated RT ± chemotherapy. Median margin dose was 14.5 Gy. Median follow-up duration was 55 mo. For the entire series, PFS after GKSRS (including tumor growth and cyst enlargement) was 91.7%, 82.8% and 70.8% at 1, 3 and 5 years, respectively. Univariate analysis revealed that solid lesion, target volume < 8 cc, newly diagnosed disease, and no brainstem involvement were prognostic factors for improved PFS with statistical significance. The authors concluded that treatment response was better in small volume residual solid juvenile pilocytic astrocytomas and GKSRS should be considered if resection is not feasible or in the presence of early recurrence[81].

Henderson *et al*[82] reported the Indiana University experience with GKSRS for low grade astrocytoma management in a series of 12 patients. Median age was 17.4 years (range: 5.9-63 years). A total of 13 lesions were treated using a median margin dose of 13 Gy. With a median follow-up duration of 48.2 mo, 2- and 4-year tumor control rates were 84.6% and 76.9, respectively. Overall survival and PFS rates were 83.3% and 75% at 4 years, respectively. The authors concluded that GKSRS could provide local control for carefully selected patients with unresectable or recurrent low grade astrocytomas[82].

Weintraub *et al*[83] from Virginia University reported outcomes of GKSRS for management of 24 pediatric patients. Median age was 11 years (range: 4-18 years). Out of the 24 patients, 15 patients were diagnosed with WHO grade I astrocytoma and 4 patients were diagnosed with WHO grade II LGG by histopathological assessment. Mean tumor volume was 2.4 cc and median margin dose was 15 Gy. Median radiological follow-up duration was 74 mo and median clinical follow-up duration was 144 mo. Complete resolution of tumor was achieved in 5 patients (21%) and ≥ 50% reduction in tumor size was achieved in 18 patients (75%). The authors concluded that GKSRS offers good clinical control of residual or recurrent gliomas in pediatric patients[83].

Hallemeier *et al*[84] reported outcomes of 18 patients (including 6 children) treated with GKSRS for recurrent or unresectable pilocytic astrocytoma at the Mayo Clinic. Median age was 23 years (range: 4-56 years). One or more prior surgical resection was performed in 13 patients (72%). Ten patients (56%) had received previous conventionally fractionated external beam RT and 4 patients (22%) had received prior systemic chemotherapy. Median treatment volume for GKSRS was 9.1 cc. Median margin dose was 15 Gy for previously irradiated patients and 16 Gy for patients without prior RT. Median follow-up duration was 8 years. PFS rates were 65%, 41%, and 17% at 1, 5, and 10 years, respectively. Overall survival rates were 94%, 71%, and 71%, at 1, 5, and 10 years after GKSRS, respectively. Prior external beam RT was found to be associated with inferior overall survival and PFS outcomes. The authors concluded that GKSRS could serve as a meaningful therapeutic option for management of recurrent or unresectable pilocytic astrocytomas when surgery and/or external beam RT fails[84].

Lizarraga *et al*[85] from the University of California reported outcomes of LINAC-based stereotactic irradiation for progressive/residual pilocytic astrocytomas in a series of 12 patients (including 5 children < 18 years of age). Median age at the start of stereotactic irradiation was 21 years (range: 5-41 years). All patients had undergone upfront partial surgical debulking as initial management without adjuvant chemotherapy or RT. Salvage stereotactic irradiation was considered in the setting of local progression. LINAC-based SRS was used to treat a median target volume of 1.69 cc in 3 patients with a median dose of 18.75 Gy. LINAC-based SRT with a median total dose of 50.4 Gy was used to treat a median target volume of 6.5 cc in 9 patients. No radiation induced adverse effects were observed in the study, and probabilities of long term PFS and disease specific survival were 73.3% and 91.7%, respectively[85].

Simonova *et al*[86] from Prag assessed long term outcomes of GK-based SRS or SRT for pilocytic astrocytomas in a series of 25 pediatric patients. Median age was 13 years (range: 3-17 years)[86]. Selection of single fraction or fractionated stereotactic irradiation was based on lesion size, location and proximity to surrounding critical structures. Median target volume was 2.7 cc (range: 0.2-25 cc). Five patients (20%) received single fraction radiosurgery with a median dose of 16 Gy. Twenty patients (80%) received stereotactic irradiation in 5 or 10 fractions using a median dose of 25 Gy. The 10-year overall survival and PFS rates were 96% and 80%, respectively. A significantly better PFS was observed in patients with a planning target volume of 2.7 cc or less. The authors concluded that radiosurgery offers an alternative therapeutic modality for management of small residual or recurrent pilocytic astrocytomas providing long term local control[86].

Trifiletti *et al*[87] reported outcomes of 28 patients receiving GK-based stereotactic irradiation for management of pilocytic astrocytomas at the University of Virginia. Median age was 17.4 years (range: 2-70.3 years). Single fraction GKSRS was performed in 27 patients, and 1 patient received stereotactic irradiation in 3 fractions. Median tumor volume was 1.84 cc and median margin dose was 16 Gy. Median clinical follow-up duration was 5.2 years and median radiological follow-up duration was 4.6 years. Local tumor control rate was 93% without adverse radiation effects. Actuarial PFS rates were 96%, 96%, 96%, and 80% at 1, 3, 6, and 12 years, respectively. The authors concluded that SRS offers an appropriate technique for management of pilocytic astrocytomas in the primary or recurrent disease setting with favorable tumor control rates and infrequent clinical toxicity[87].

Gagliardi *et al*[88] assessed long term outcomes of GKSRS for LGG. Their series of 39 patients included 10 pediatric patients. Median age was 31 years (range: 9-72 years). Most common histology was pilocytic astrocytoma. Median tumor volume was 1.24 cc. Median margin dose was 15 Gy. Median follow-up duration was 54.5 mo. Actuarial PFS rates at 1, 5, and 10 years were 74.9%, 52.8%, and 39.1%, respectively. Assessment of patients' quality of life and functional performance was performed by utilization of standardized functional performance scores and validated subjective health survey questionnaires. Clinical improvement and Karnofsky Performance Status improvement were observed in 52.4% and 45.5% of the patients, respectively. The authors concluded that GKSRS may serve as a viable therapeutic modality for management of LGG which may provide tumor growth control and improve patients' functional performance and quality of life with optimization of social functioning and minimization of disease-related psychological impact[88].

In summary, stereotactic irradiation has been more frequently incorporated into management of pediatric LGG as compared to adult HGG. Pilocytic astrocytoma accounts for the majority of pediatric LGG and may be considered as suitable for radiosurgical treatment with its well-defined borders on neuroimaging. Clearly, several other factors are critical in decision making for stereotactic irradiation of a pediatric patient with LGG. Stereotactic irradiation has been used as primary therapy in the presence of deeply seated lesions at eloquent brain areas, or as a boost treatment in conjunction with conventionally fractionated external beam RT, and more frequently to treat progressive or recurrent pediatric LGG (Table 1)[73-88]. Overall, these series reported favorable tumor control rates with stereotactic irradiation. Improvements have been observed in clinical symptoms, functional performance and quality of life parameters with low rates of severe toxicity. However, there is still room for improvement with the need for accumulation of further robust and high level evidence to consider stereotactic irradiation as a standard part of management for pediatric LGG.

CONCLUSION

Pediatric brain tumors are the most common solid tumors in children which may lead to morbidity and mortality. Gliomas comprise the majority of brain tumors in children. Radiotherapeutic management of gliomas in children poses a formidable challenge considering the adverse effects of irradiation for this vulnerable patient population. In this context, efforts have been focused on improving the toxicity profile of radiation delivery. Stereotactic irradiation with SRS or SRT in a single or few treatment fractions may serve as a viable radiotherapeutic approach to achieve this goal. High conformality along with steep dose gradients around the target volume allows for reduced normal tissue exposure under precise immobilization and image guidance. While conventionally fractionated RT regimens administered over 5 wk to 6 wk may lead to

substantial burden on children particularly when daily anesthesia is needed, radiosurgical approaches allow for abbreviated treatment courses. Also, margin-free strategies may be considered in the setting of stereotactic irradiation with precise immobilization and image guidance for management of well demarcated lesions such as pilocytic astrocytomas[89].

Overall, stereotactic irradiation has been utilized less frequently for HGG and more commonly for LGG in children[58-61,73-89]. Some of the studies reporting data on stereotactic irradiation of pediatric gliomas also included adult patients. Drawing firm conclusions may be confounded by diversities in patient, tumor, and treatment characteristics in studies with limited number of patients and inherent limitations. Nevertheless, available limited data on stereotactic irradiation of pediatric gliomas suggest potential utility of this contemporary approach as part of initial management or for treatment of progressive or recurrent lesions despite the need for further supporting evidence.

In the context of future directions, immunotherapy, identification of driver alterations and introduction of effective targeted therapies may pave the way for innovatory treatment strategies for children with pediatric glial neoplasms[90-93]. There is need for active investigation on development of safe and efficacious therapeutic approaches for management of pediatric glial neoplasms.

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MINIREVIEWS

Rationalising animal research synthesis in orthopaedic literature

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Abstract

Systematic reviews in orthopaedic literature are frequently criticised for offering inconsistent conclusions. On top of that, high-quality randomized human evidence on crucial orthopaedic topics is more often than not lacking. In this situation, pooling animal literature could offer an excellent insight into unanswered critical clinical questions, thus potentially improving healthcare. In this paper, we sought to present the rationale and basic principles governing meta-analysis of animal research. More specifically, we elaborated on the available evidence-based methods to achieve a scientifically sound animal data synthesis. In addition, we discussed result interpretation, strength of recommendations and clinical implications based on the results of these meta-analytic modalities.

Key Words: Meta-analysis; Animal research; Evidence synthesis; *in vivo*; Orthopaedics

Core Tip: Relying on the findings of properly conducted meta-analyses of animal research is crucial, particularly in the paucity of human evidence on crucial orthopaedic topics. It is an undeniable fact that authors tend to encounter a great many challenges when conducting this type of research as they have to address several potential sources

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of bias. For that reason, we advocate that readers should critically appraise the findings of animal syntheses papers.

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INTRODUCTION

Given the nature and rarity of many orthopaedic diseases, conducting high-quality double-blind randomized control trials is not always feasible. This is particularly true when it comes to addressing a particular orthopaedic surgical intervention. Hence, crucial research questions remain unanswered due to the fact that safe conclusions cannot be drawn purely based on a few underpowered and low-quality individual studies. In this situation, animal evidence could offer valuable information towards delineating the potential of a prevention and/or therapeutic orthopaedic intervention.

The rationale behind synthesizing animal literature is to avoid the potential bias which is commonly detected in narrative literature reviews. To elaborate, selective presentation of individual study findings and incorrect weighting of conclusions can exert a negative impact on the credibility of a systematic review. Rather, by summarizing the results of multiple individual studies a researcher could potentially produce more valid results provided that guidelines governing meta-analyses of animal papers are respected. In this paper, we sought to present the key elements for conducting a high-quality meta-analysis of animal research which could provide a useful insight into unanswered clinical questions in orthopaedics.

PROSPECTIVE ANIMAL REVIEW REGISTRATION AND REPORTING GUIDELINES. ARE THEY NECESSARY?

Regardless of the nature of the subjects utilised in an *in vivo* evidence synthesis, it is strongly advocated that systematic reviews be prospectively registered with a valid database (*e.g.*, PROSPERO). The main reason behind this protocol registration is to increase transparency in reporting and prevent selective outcome reporting issues.

On top of that, abiding by published guidelines for systematic reviews (*e.g.*, Preferred Reporting Items for Systematic Reviews and Meta-Analyses) is of utmost importance given the fact that poor reporting diminishes accuracy and potential usefulness of an animal meta-analysis[1].

CONTROLLED *VS* UNCONTROLLED DATA SYNTHESIS: IS THERE ANY DIFFERENCE?

From a methodological standpoint, if properly controlled homogenous groups are available, then standard head-to-head meta-analysis can be safely undertaken by using a readily available piece of statistical software [*e.g.*, Review Manager (RevMan)][2]. However, synthesising uncontrolled research represents a different task which can be achieved by means of proportional meta-analysis[3]. It is underlined that although indirect comparisons could be made by comparing overlapping of confidence intervals in the aforementioned type of meta-analysis, safe conclusions on the comparative efficacy of interventions cannot be reached and therefore this approach is not generally recommended.

LUMPING INTERVENTION GROUPS IN META-ANALYSES OF ANIMAL RESEARCH

One frequently encountered methodological issue in pair-wise meta-analyses is the limited statistical power precluding reliable conclusions to be drawn[4]. To address this issue, lumping intervention groups into valid subgroups with respect to literature classifications[5,6] is recommended. By and large, a crucial point authors need to pay attention to when they elect for the subgroup pathway is the trade-off between statistical power and precision in reporting. We advocate that as long as published guidelines have been followed prior to creating subgroups and sensitivity analysis has been conducted to investigate the impact of subgrouping on the data synthesis, the validity of the findings is not severely compromised.

POOLING DICHOTOMOUS AND CONTINUOUS DATA MEASURING THE SAME OUTCOME. IS IT POSSIBLE?

Encountering a situation where information for the same outcome is presented in some studies as dichotomous data and in other papers by means of a continuous variable is a common phenomenon in animal research. To address this issue, re-expressing standardized mean differences to odds ratios (or the *vice versa*) is recommended[7]. Subsequently the generic inverse variance model in RevMan can be utilised to pool those converted data together[7] (Figure 1). Although we recognise this could be a challenging task for a researcher to accomplish, the problem of missing information which may compromise the validity of the meta-analysis results can be overcome[5].

FEASIBILITY OF EXTRACTING QUANTITATIVE DATA FROM GRAPHICAL PRESENTATIONS

Meticulous data extraction is a crucial element in performing a satisfactory systematic review and meta-analysis. It is a common phenomenon in original papers published a long time ago to present their findings in a graphical manner with no corresponding numerical data. In this situation, taking advantage of the use of an appropriate software tool (*e.g.*, Plot Digitizer and Getdata Graph Digitizer)[8] which allows for reliable digitization of graphs and/or plots is recommended to abstract and subsequently synthesise the required information.

QUALITY ASSESSMENT IN SYSTEMATIC REVIEWS OF ANIMAL PAPERS

Quality appraisal of individual animal studies performed by means well-established tool such as the SYRCLE's Risk of Bias tool[9], ensures consistency and prevents discrepancies in assessing risk of bias in systematic reviews of animal intervention studies. SYRCLE's Risk of Bias tool is an adaptation of the Cochrane Risk of Bias tool which could potentially facilitate transition of animal research into clinical practice. On top of that, due to the relatively standardised use of this instrument in the existing literature, the necessity of improving particular methodological aspects of animal studies can be easily stressed[9]. It should be noted that a graphical quantification of the risk of bias summarising the assessments for each domain could be of essence (Figure 2)[5].

IS PUBLICATION BIAS A COMMON THREAT TO VALIDITY IN LABORATORY ANIMAL RESEARCH?

It is an undeniable fact that "negative" laboratory animal results more often than not remain unpublished[10]. Therefore, exploration of selective reporting in animal papers appears to be critical. In other words, merely relying on statistical significance may introduce bias in the results of the statistical analysis and potentially threaten the validity of the meta-analysis findings.

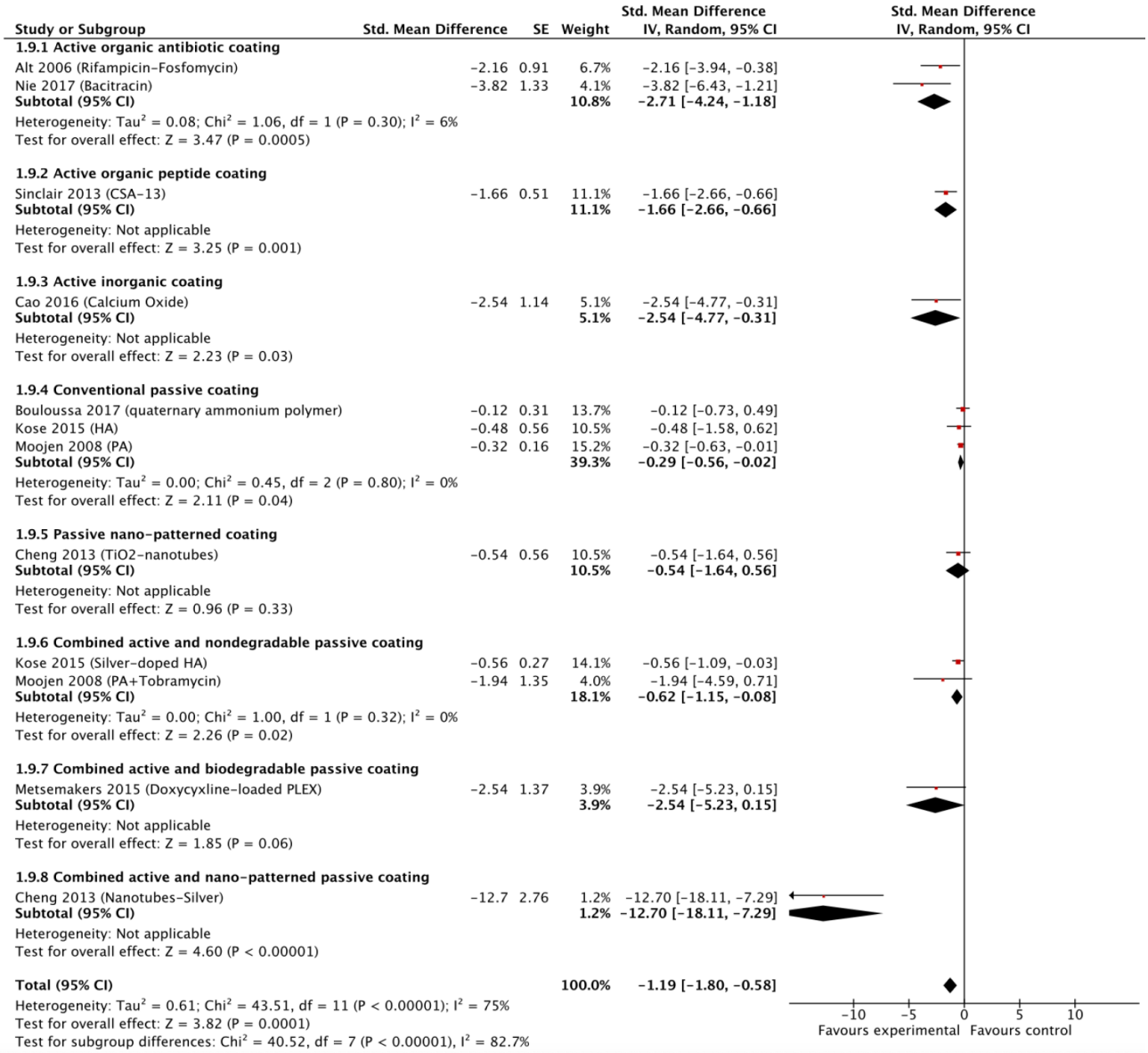

Figure 1 Forest plot of standardised mean differences with multiple subgroup analyses is demonstrated. The methicillin-resistant *Staphylococcus aureus* infection prevention potential is assessed by means pair-wise meta-analysis in inverse variance mode to consider not only continuous but also dichotomous data in the analysis. CI Confidence interval; CSA: Cationic steroidal antimicrobial; HA: Hydroxyapatite; SMD: Standardised mean difference; IV: Inverse variance; PA: Periapatite; PLEX: Polymer-lipid encapsulation matrix; SE: Standard error; TiO2 = Titanium dioxide. Citation: Tsikopoulos K, Sidiropoulos K, Kitridis D, Hassan A, Drago L, Mavrogenis A, McBride D. Is coating of titanium implants effective at preventing *Staphylococcus aureus* infections? A meta-analysis of animal model studies. *Int Orthop* 2020. Copyright© The Author(s) 2020. Published by Springer Nature Publishing Group[5]. The authors have obtained the permission for figure using from the Springer Nature Publishing Group (Supplementary material).

HIERARCHY OF EVIDENCE-BASED MEDICINE AND BIAS ASSESSMENT

It is highlighted that while a systematic review is generally better than an individual study, a meta-analysis of animal studies should not be placed at the top of the hierarchy in a pyramid that depicts validity[11]. This is because a meta-analysis is as good as the studies identified and included[12]. Nevertheless, in the absence of high-quality evidence, relying on the results of a meta-analysis of animal models is advisable provided that caution is exercised due to potential bias.

INTERPRETING RESULTS AND DRAWING CONCLUSIONS

It is worthy of note that prior to drawing meta-analysis conclusions, sample size of the included comparison groups, quality rating of the involved studies, effect sizes, and statistical heterogeneity should be taken into account. On top of that, investigating the

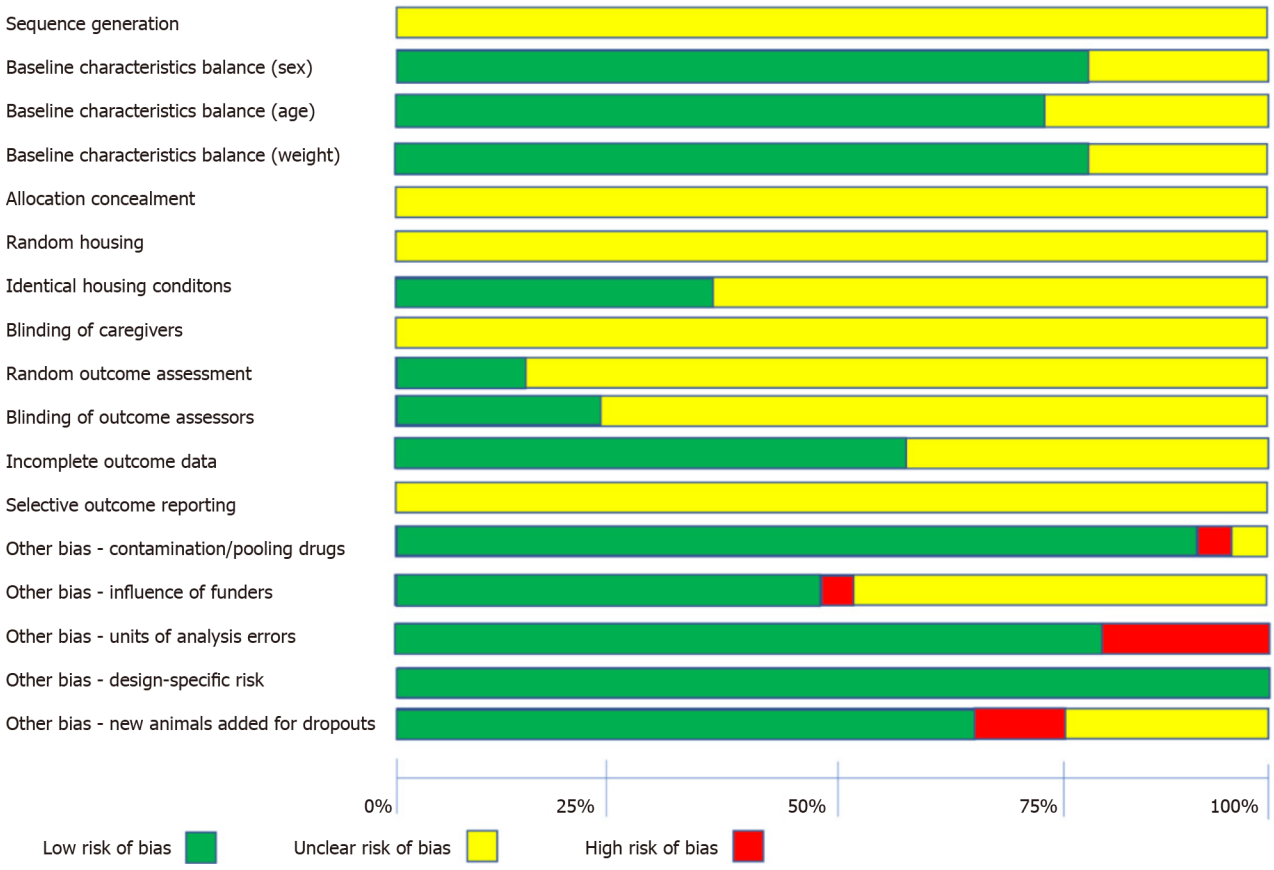

Figure 2 Quantification of risk of bias assessment enables not only summarising quality appraisal results but also making judgments as to what the future studies should look at. Citation: Tsikopoulos K, Sidiropoulos K, Kitridis D, Hassan A, Drago L, Mavrogenis A, McBride D. Is coating of titanium implants effective at preventing *Staphylococcus aureus* infections? A meta-analysis of animal model studies. *Int Orthop* 2020. Copyright© The Author(s) 2020. Published by Springer Nature Publishing Group[5]. The authors have obtained the permission for figure using from the Springer Nature Publishing Group (Supplementary material).

impact of various sources of clinical heterogeneity by means of a sensitivity analysis (*i.e.*, exclusion of one or more papers from the analysis to assess the impact of a particular confounding factor on the findings of the study) with a view to verify the meta-analysis results is strongly advocated.

CONCLUSION

Despite the abundance of literature on developing meta-analytic skills relating to human data, methodological papers dealing with animal data synthesis are lacking. In the current article, we focused on the technicalities and implications of pooling animal literature which could be particularly useful when investigating the results of orthopaedic surgical interventions in the absence of human evidence. It is worthy of note that due to the experimental nature of animal papers, a certain amount of uncertainty in the meta-analysis conclusions is anticipated. For that reason, we advise caution when it comes to extrapolating the results of this type of research back to human biology.

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MINIREVIEWS

Bowel intussusception in adult: Prevalence, diagnostic tools and therapy

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Abstract

Intussusception is defined as invagination of one segment of the bowel into an immediately adjacent segment. The intussusception refers to the proximal segment that invaginates into the distal segment, or the intussusception (recipient segment). Intussusception, more common occur in the small bowel and rarely involve only the large bowel. In direct contrast to pediatric etiologies, adult intussusception is associated with an identifiable cause in almost all the symptomatic cases while the idiopathic causes are extremely rare. As there are many common causes of acute abdomen, intussusception should be considered when more frequent etiologies have been ruled out. In this review, we discuss the symptoms, location, etiology, characteristics, diagnostic methods and treatment strategies of this rare and enigmatic clinical entity in adult.

Key Words: Adult intussusception; Bowel invagination; Bowel obstruction; Computed tomography; Laparoscopic surgery; Endoscopy

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Core Tip: Intussusception in adult is rare, but its onset is often tumor-related. The diagnosis of intussusception in adult is challenging as a result of the nonspecific signs and symptoms. We herein discuss the epidemiology and the clinical features of bowel intussusception in adult and the role of radiology and surgery in the management of this insidious condition.

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INTRODUCTION

The term intussusception refers to the invagination of a segment of the gastrointestinal tract into the lumen of an adjacent segment[1]. This condition lead to a transient or permanent bowel obstruction that can evolve even to intestinal ischemia. Intussusception is much more prevalent in children rather than in adult with an overall incidence in the second group of around 2-3 cases per million of the general population per-year[2].

Adult intussusceptions often onsets as an intermittent cramping abdominal pain associated with signs of bowel obstruction[3]. Diagnosis of intussusception in adult is challenging since the acute abdominal pain is at the same time a non-specific symptom and one of the most frequent complaint reported in the setting of emergency medicine.

Past medical history, physical exam and laboratory test can aid to increase the level of suspicion, but imaging is almost always needed to make diagnosis of bowel intussusception. Although abdominal computed tomography (CT) scan is useful in this setting, it has low specificity in differentiate malignant, benign or idiopathic lead points[4-6].

The optimal management for adult intussusception is still controversial, nevertheless its definitive treatment consists in surgical intervention with appropriate approach depending on the underlying etiology and location.

ETIOLOGY

Any perturbation of the normal pattern of intestinal peristalsis increase the risk of intussusception[7]. As opposed to the pediatric population, adult intussusception is commonly caused by a pathologic lead point; it can be located in the lumen of the bowel, inside the wall or extramural[8], and its occurrence is associated to an identifiable cause in 80%-90% of symptomatic cases[7,9,10]. The causes of adult intussusception are summarized in Table 1.

Malignant and benign neoplasms account for 60% of cases with a lead point; the remaining non-idiopathic cases are usually caused by postoperative adherences, Crohn's disease, infections, intestinal ulcers, and Meckel diverticulum[7,11].

In a recent systematic review and meta-analysis from Hong *et al*[12] 1229 adults with intussusception were identified from 40 retrospective case series: Pooled rates of malignant and benign tumors and idiopathic etiologies were 32.9%, 37.4% and 15.1%, respectively.

According to several reports[7-9,11], when dividing etiologies by enteric and colonic location, the small bowel intussusception is more often caused by benign lesions. In contrast, colonic intussusception is more likely to have an underlying malignant lead point (often a colonic adenocarcinoma). When the small bowel intussusception is induced by malignant lesions these are often metastatic disease (*i.e.*, carcinomatosis).

Notably the ileocolic location in adult intussusception is a variant in which almost the totality of cases has a malignant lead point involving the ileocecal valve[9] (Table 2).

Table 1 Causes of adult intussusception

Benign	Malignant
Enteric	
Adherences, coeliac disease, Crohn's disease, endometriosis, hamartoma, infections, Kaposi sarcoma, lipoma, Meckel diverticulum, neurofibroma, polyps (inflammatory, adenomatous), stromal tumor, tubercolosis	Adenocarcinoma, carcinoid tumors, leiomyosarcoma, lymphoma, malignant gastrointestinal stromal tumor, metastatic carcinoma, neuroendocrine tumor
Colonic	
Adherences, inflammatory pseudopolyp, lipoma, polyps (inflammatory, adenomatous)	Adenocarcinoma, lymphoma, sarcoma

Table 2 Frequent causes of adult intussusception located to ileocolic site

Ileocolic	
Malignant	Adenocarcinoma, metastatic carcinoma, lymphoma, gastrointestinal stromal tumor

PREVALENCE

As previously mentioned, bowel intussusception afflicts children more than adults with an approximate ratio of 20 to 1. In fact intussusception in adult account for < 5% of all cases of intussusception and is found in 1% of patients with bowel obstruction[7], with a surgical report of less than 1 in 1300 abdominal operations[13]. Usually it involves adults, after the fifth decade, with no difference among male and female[8].

The bowel intussusception is commonly classified in four types according to the land-marks of its origin and extension: (1) Enteric type: the intussusception is limited to the small intestine; (2) Ileocolic type: the ileum passes the ileocolic segment, but the appendix does not invaginate; (3) Ileocecal type: the ileocecal portion invaginates into the ascending colon; and (4) Colocolonic type: the intussusception is limited to the colon and rectum (no anal protrusion).

Small bowel is more often involved by intussusception rather than large bowel. Based on the systematic review of Hong *et al*[12] the pooled rates of enteric, ileocolic, and colonic location types account for 49.5%, 29.1%, and 19.9%, respectively.

This predominance of enteric intussusception has its exception in the populations of the central and western Africa in which is most common the cecocolic intussusception (tropical intussusception)[14] probably for the interaction of dietary habits (high-fiber diet), genetics and gut microbiome features.

PATHOPHISIOLOGY AND CLINICAL PRESENTATION

The most common locations involved in intussusception are at the junctions between mobile and fixed segments of the bowel, such as between the freely-moving ileum and the retroperitoneal cecum[8].

Part of a proximal segment of the bowel slides into the next distal section. This event can lead to bowel obstruction and intestinal ischemia. The compromised blood flow to the affected segment can cause necrosis of the intestinal wall with bacterial translocation, peritonitis, sepsis and even perforation. The clinical scenario can be variable but usually characterized by acute intermittent or constant crampy abdominal pain, vomiting and bloating[15,16].

Pain is the most common symptom reported at a rate of up to 80% in several series[11,12,17,18].

The patient present abdominal tenderness and signs of systemic inflammatory response syndrome (*i.e.*, hypothermia or hyperthermia, hypotension, and tachycardia). Fever is usually a sign of the onset of intestinal necrosis. Decreased or absent bowel sounds can be present as well as signs of parietal peritoneal irritation, failure to gas passage, abdominal masses, and diarrhoea even with bloody stool. Laboratory tests usually document increase of leukocytes count and inflammatory markers such as polymerase chain reaction.

DIAGNOSTIC TOOLS

As already extensively presented in previous studies, the preoperative diagnosis of bowel intussusception raises several questions to the doctor and in this regard, a paper published by Reijnen *et al*[19] report a preoperative diagnostic rate of 50%.

Intestinal intussusception presents considerable variability in the patient's clinical presentation (abdominal pain, vomiting, nausea) and shows signs of palpable abdominal masses on objective examination.

To make a correct differential diagnosis with other similar intestinal pathologies, it is therefore useful to use radiodiagnostic instruments: abdomen X-ray, small bowel series with barium, abdominal ultrasound, abdominal CT.

Intussusceptions are classified according to location (enteroenteric, ileocolic, ileocecal, or colo-colic) and cause (benign, malignant, or idio-pathic).

The abdomen X-ray (Figure 1) may reveal signs of intestinal obstruction (hydro-air levels, distension of the intestinal tract upstream, unexplained masses) which can occur in different abdominal quadrants depending on the level of obstruction (high or low)[20].

An upper gastrointestinal contrast entero-X-ray may show a "stacked coin" or "coil-spring" appearance, while the lower gastrointestinal contrast entero-X-ray, useful in patients with colic or ileus-colic obstruction, may show a “cup-shaped” filling defect or “spiral” or “coil-spring” appearances[20].

Another useful tool is ultrasound, a methodical operator dependent, which can show signs such as the “target” or “doughnut” in the transverse scans (Figure 2), or the "pseudo-kidney" sign or "hay-fork" sign in the longitudinal view[21].

CT is currently considered the gold standard for the intussusception diagnosis. Very sensitive, it can highlight the position, the nature of the mass and the relationship with the surrounding tissues[20].

The CT scan may help to find a lead-point intussusception that can be localized in the all bowel tract. The CT scan can also demonstrate some pathognomonic radiological aspects as target-like and the sausage-shaped soft tissue mass. These specific findings can be clearly visible or they can remain undetected due to edema; in these cases the classic three-layer appearance and anatomic detail are often lost and so an irregularity mass can show the intussusception. Mesenteric fat and blood vessels are barely visible.

THERAPY

As previously reported, adult’s intussusception is frequently cause by a pathologic lead point. For those reasons, treatment of bowel intussusception causing obstruction has typically involved surgery, often with bowel resection, as opposed to the pediatric population. The attempt of hydrostatic reduction in the adult population is not indicated; on the contrary, in the pediatric population this is the treatment of choice in the majority of cases; in fact, in this latter group of age the percentage of surgical treatment is so far less the 10% of the reported cases[22].

In recent series and retrospective review articles[23-26], the evidence that the increased use of cross sectional imaging such as CT has resulted in increment of the radiological diagnosis of intussusception, with a successful nonoperative management in many cases, has led to some degree of controversy regarding optimal management of these patients.

The main issues in the management of adult intussusception are: (1) When proceed with surgical exploration; (2) Once the surgical approach is the treatment of choice, whether attempt intraoperatively reduction or proceed direct to resection of the affected segments; and (3) Once the surgical approach is the treatment of choice, it should be performed open or laparoscopically.

In the most recent review article is reported that surgical exploration is the treatment of choice in case of: (1) Patients with signs and symptoms of acute abdomen; in this scenario abdominal exploration is the gold standard when symptoms of clinical obstruction are reported in association with radiological signs of obstruction, dehydration and increase of white blood cells along with inflammatory markers at laboratory tests; emergency exploration is mandatory in presence of signs of septic shock and peritonism (conditions almost always suggestive of intestinal ischemia); (2) Patients with diagnosis of intussusception with a mass visible on CT scan, also in the absence of clear clinical signs of acute abdomen; and (3) Patients with diagnosis of colonic or ileocolic intussusception, usually associate with neoplasm, also in the

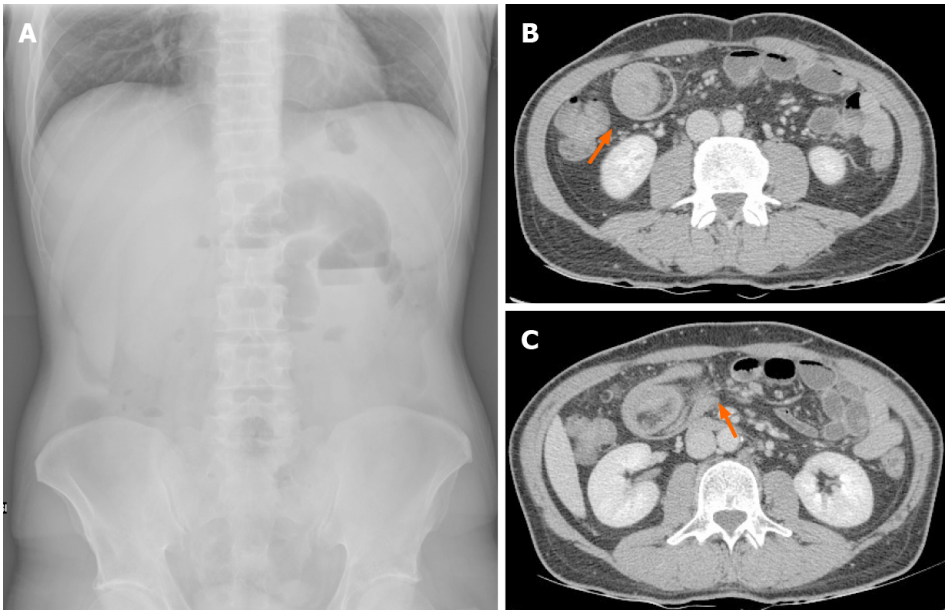

Figure 1 Inflammatory fibroid polyp of the small intestine. A 43-year-old male presenting with abdominal pain and vomiting. A: Abdomen X-ray showed signs of intestinal obstruction with hydro-air levels in the upper quadrants; B: Computed tomography scan confirmed bowel obstruction with presence of "target sign" (orange arrow); C: Mesenteric fat and blood vessels are visible (orange arrow). Surgical resection revealed an inflammatory fibroid polyp of the ileum.

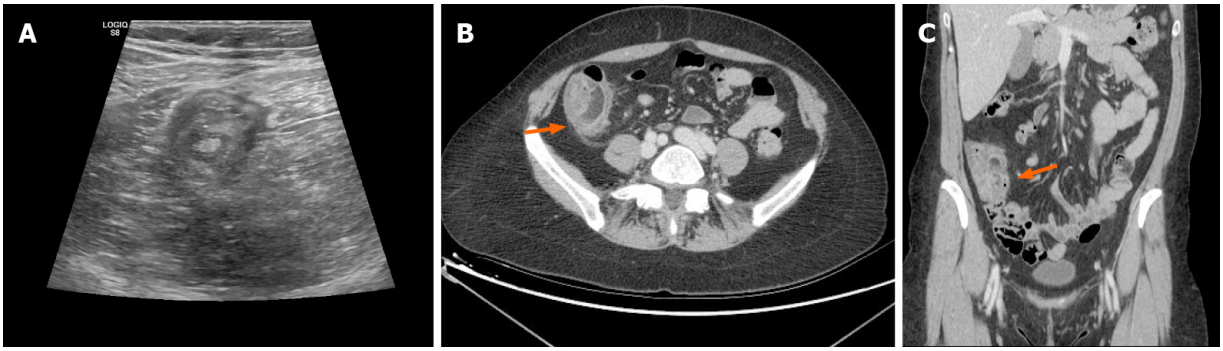

Figure 2 Ileocecal valve adenocarcinoma. A 56-year-old female presenting with right iliac fossa pain. A: Ultrasound scan revealed "target sign"; B and C: Computed tomography scan confirmed ielo-colic intussusception, with no signs of bowel obstruction [orange arrow, horizontal (B) and coronal (C)]. Surgical resection revealed an ileocecal valve adenocarcinoma (pT2 N0).

absence of clear clinical signs of acute abdomen. In these setting, preoperative endoscopy can be done in order to confirm the presence of pathology and/or cancer[8].

On the other side, many reports suggest a "wait and see" strategy, with serial clinic and imaging evaluation to ensure spontaneous resolution in entero-enteric intussusceptions without lead point mass and short affected segment (< 3.5 cm)[24-26]. Based on the systematic review of Hong *et al*[12], it is important to remark that the pooled rate of patients that received this type of conservative treatment is less of 5% and is limited to patient with entero-enteric locations.

Undoubtedly, other controversy remains as to whether reduction of the intussusception should be attempted intraoperatively[27,28].

This controversy is related to the consideration that reducing the intussusception before resection carries risks of perforation and the theoretical possibility of dissemination of malignant cells during the attempt. The other theoretical risks of preliminary manipulation and reduction of an intussuscepted bowel is related to the endangerment of anastomotic complications of the manipulated friable and edematous bowel tissue[16,20].

On the other hand, the reduction of bowel intussusception is useful both to preserve important lengths of small bowel and to prevent possible development of short bowel syndrome, especially when the small bowel is the only tract involved because of its lower rate of association to malignancy[29,30].

On this point, we suggest that simple reduction is acceptable in post-traumatic or idiopathic intussusceptions, where no pathological cause could be identified, obviously after the exclusion of bowel ischaemia or perforation, especially in case of small bowel intussusception. Considering the high rate of primary adenocarcinoma, colonic intussusception should be resected *en bloc* without reduction to avoid potential intraluminal seeding or venous tumor dissemination: a formal resection using appropriate oncologic techniques are recommended, with the construction of a primary anastomosis between healthy and viable tissue. Finally, a selective approach seems appropriate for ileocolic adult intussusception because of its intermediate nature between enteric and colonic sites[11,12,31].

The choice of preforming laparoscopic rather than open procedure depends both on the clinical condition of the patient and on surgeon's laparoscopic experience[8,27].

A standardized laparoscopic technique to approach intussusception is not available, due to the all different possible causes and locations, some tips and tricks are reported in literature[8,32,33]: the pneumoperitoneum establishment must be performed with open laparoscopy at the umbellicum because of the high risk of bowel lesions with the Verres technique. Due to the rarity of the left-side's intussusception is recommend to place the two additional 5-mm ports one in the left lower quadrant and the other suprapubically. If needed, other ports can be placed depending on the location of the pathology. During laparoscopy all four quadrants of the abdomen and the pelvis must be thoroughly explored; once the pathologic segment is found, it can either be resected or eviscerated and dealt with extracorporeally using small incision, depending on surgeon skill and severity of the occlusive syndrome related to intussusception. It is recommended to sample suspected fluid collections for culture as well as to biopsy suspected lesions.

CONCLUSION

Bowel intussusception in adult is a rare condition with acute onset or seldom-elusive progress. Clinicians and surgeons are not supported by designated scoring systems in this challenging diagnosis because of non-specific symptoms, and its preoperative identification is often missed or delayed. On the other hand, intussusception is a surgical emergency associated to high rates of mortality in case of delayed treatment, therefore it is important to think about this less common diagnostic possibility when facing an acute abdominal pain with sign of bowel obstruction.

The management of bowel intussusception in adult remains mainly surgical. The timing and type of approach depends on several factors such as the underlying causes, the severity of clinical presentation, the site and the length and vitality of the bowel segment involved.

Anyway, the increased use of cross sectional imaging has increased the early-diagnosis of intussusception, in many cases with a successful nonoperative management; such findings led to some questioning about the optimal management of these conditions.

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ORIGINAL ARTICLE

Randomized Clinical Trial

Comparison of lag screws and double Y-shaped miniplates in the fixation of anterior mandibular fractures

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ORCID number: Lydia Melek 0000-0001-6379-3800.

Author contributions: Melek L solely contributed to the design of the study, implementation of the clinical trial, writing and revision of the manuscript.

Institutional review board statement: This study was approved by the Institutional Review Board (IRB) of the Faculty of Dentistry, Alexandria University, Egypt, and the protocols used in the study were approved by the Research Ethics Committee. Human Subjects Review: Approval Number: IRB 00010556-IORG 0008839 Board Name: Research Ethics Committee, Alexandria Faculty of Dentistry Board Affiliation: Faculty of Dentistry, Alexandria University, Egypt Phone: (+203) 4812201.

Clinical trial registration statement: The study was registered on clinicaltrials.gov (ClinicalTrials.gov ID: NCT04396054).

Informed consent statement: A written informed consent was signed by each patient before the operation.

Conflict-of-interest statement: The author declares no conflict of interest.

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Abstract

BACKGROUND

Mandibular fractures constitute about 80.79% of maxillofacial injuries in Alexandria University, either as isolated mandibular fractures or as a part of panfacial fractures. The combination of symphyseal and parasymphyseal fractures represent 47.09% of the total mandibular fractures.

AIM

To compare the effectiveness of lag screws *vs* double Y-shaped miniplates in the fixation of anterior mandibular fractures.

METHODS

This study is a prospective randomized controlled clinical trial, performed on sixteen patients with anterior mandibular fractures. Patients were divided equally into two groups, each consisting of eight patients. Group 1: Underwent open reduction and internal fixation using two lag screws. Group 2: Underwent open reduction and internal fixation using double Y-shaped plates. The following parameters were assessed: operating time in minutes, pain using a visual analog scale, edema, surgical wound healing for signs and symptoms of infection, occlusion status and stability, maximal mouth opening, and sensory nerve function. Cone beam computed tomography was performed at 3 and 6 mo to measure bone density and assess the progression of fracture healing.

RESULTS

The study included 13 males (81.3%) and 3 females (18.8%) aged 26 to 45 years (mean age was 35.69 ± 6.01 years). The cause of trauma was road traffic accidents in 10 patients (62.5%), interpersonal violence in 3 patients (18.8%) and other causes in 3 patients (18.8%). The fractures comprised 10 parasymphyseal fractures (62.5%) and 6 symphyseal fractures (37.5%). The values of all parameters were comparable in both groups with no statistically significant difference except for the mean bone density at 3 mo postoperatively which was 946.38 ± 66.29 in group

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1 and 830.36 ± 95.53 in group 2 (P = 0.015).

CONCLUSION

Both lag screws and double Y-shaped miniplates provide favorable means of fixation for mandibular fractures in the anterior region. Fractures fixed with lag screws show greater mean bone density at 3 mo post-operation, indicative of higher primary stability and faster early bone healing. Further studies with larger sample sizes are required to verify these conclusions.

Key Words: Anterior mandibular fractures; Symphyseal fracture; Parasymphyseal fracture; Miniplates; Lag screws; Double Y-shaped plates

Core Tip: The aim of this study is to compare the effectiveness of lag screws *vs* double Y-shaped miniplates in the fixation of anterior mandibular fractures in terms of fracture stability and progression of bone healing.

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INTRODUCTION

Mandibular fractures constitute about 80.79% of maxillofacial injuries in Alexandria University, either as isolated mandibular fractures or as a part of panfacial fractures. The combination of symphyseal and parasymphyseal fractures represent 47.09% of the total mandibular fractures[1]. However, this percentage of anterior mandibular fractures in relation to other mandibular fractures is variable among different studies and locations[2].

Lag screws have been described as a reliable, stable and safe method of internal fixation for anterior mandibular fractures. The absence of anatomical hazards, thickness of the bone cortices and curvature of the anterior mandible are all factors contributing to the suitability and success of using lag screws in that region[3].

Miniplates have been widely used for decades for the fixation of mandibular fractures owing to their easy handling and adaptation, in addition to providing functionally stable fixation[4]. Different designs of miniplates, varying from the conventional form by Champy, have been proposed to provide extra stability of the fracture. A biomechanical study has shown that double Y-shaped miniplates provide greater resistance to displacement in comparison to conventional straight miniplates[5].

The aim of this study is to compare the effectiveness of lag screws *vs* double Y-shaped miniplates in the fixation of anterior mandibular fractures.

MATERIALS AND METHODS

Ethic statements

This study is a prospective randomized controlled clinical trial. It was performed on sixteen patients with anterior mandibular fractures, selected from those admitted to the Emergency Department of Alexandria University Hospital. This study followed the Declaration of Helsinki with regard to medical protocol and ethics, and the regional Ethical Review Board of the Faculty of Dentistry, Alexandria University approved the study (Approval Number: IRB 00010556-IORG 0008839). The study was registered on clinicaltrials.gov (ClinicalTrials.gov ID: NCT04396054). A written informed consent was signed by each patient before the operation.

Patients

The patients were divided equally into two groups, each consisting of eight patients. Assignment of each patient into one of these two groups was carried out using computer random numbers: Group 1: Underwent open reduction and internal fixation using two lag screws; Group 2: Underwent open reduction and internal fixation using double Y-shaped plates.

Inclusion criteria

Patients of both genders aged from 25 to 45 years, suffering from anterior fractures of the mandible (symphyseal or parasymphyseal) were included. Those with old fractures, infected or comminuted fractures were excluded from the current study.

Study design

A thorough clinical examination was performed preoperatively on all patients, in addition to panoramic radiographs. All patients were operated by the same surgeon under general anaesthesia with nasotracheal intubation. Complete disinfection of the oral cavity and face was performed using povidone iodine solution, followed by draping with sterile towels exposing the surgical site. Maxillomandibular fixation was carried out to adjust the occlusion using arch bars and eyelet wiring. After that, an intraoral mandibular vestibular incision was made exposing the fracture line where reduction of the two segments was carried out under direct vision.

In the first group, fixation of the reduced segments was achieved using 2 lag screws (O and M Medical GmbH Eschenweg, Germany). The diameter of the screws was 2.7 mm and the length ranged from 18 to 24 mm. Screw fixation was performed by passage of the screw through a larger gliding hole into a smaller traction hole on each side of the fracture (Figure 1). In the second group, fixation of the reduced segments was achieved using double Y-shaped plates (Stryker-Leibenger, Germany) with 6 monocortical 2.0 mm diameter screws (Figure 2).

After direct fixation was performed in both groups, the incision was closed using layered suturing and the maxillomandibular fixation was removed. Postoperative care for all patients included the following: (1) Each patient received intravenous Cefotaxime 1 mg/12 h (Cefotax, by EIPICO) for one day postoperatively followed by Amoxicillin clavulanate (Augmentin, manufactured by MPU) 1 mg given orally twice daily for the next 5 d; (2) An analgesic anti-inflammatory drug in the form of Diclofenac Sodium (Rheumafen, by GlaxoSmithKline) 75 mg vial up to the second postoperative day was given followed by Diclofenac Potassium (Rheumafen tablets, by GlaxoSmithKline) 50 mg tablets three times daily for the next 5 d; (3) All patients were instructed to use chlorohexidine mouth wash (Hexitol, by an Arabic drug company) for maintenance of good oral hygiene; and (4) Instructions for a soft high calorie diet was given to all patients to be followed for 4 wk postoperatively.

Postoperative follow-up: Patients were followed up on the second, third postoperative days, first and second weeks, then after one, 3 and 6 mo. The following parameters were assessed: operating time in minutes, pain using a visual analog scale, edema, surgical wound healing for signs and symptoms of infection, occlusion status and stability, maximal mouth opening, and sensory nerve function using a dental probe to assess sensory changes along the mental nerve distribution and comparing it to the contralateral side. Cone beam computed tomography was performed at 3 and 6 mo to measure bone density and assess the progression of fracture healing.

Statistical analysis

Data were fed to the computer and analyzed by the appropriate statistical tests using the IBM Statistical Package for Social Science software version 21.0. Significance of the obtained results was set at the 5% level. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean and standard deviation. The independent samples *t*-test was used to compare the means of quantitative data.

RESULTS

This study was conducted on 16 patients suffering from anterior mandibular fractures. The study included 13 males (81.3%) and 3 females (18.8%) aged 26 to 45 years (mean age was 35.69 ± 6.01 years). The cause of trauma was road traffic accidents in 10 patients (62.5%), interpersonal violence in 3 patients (18.8%) and other causes in 3

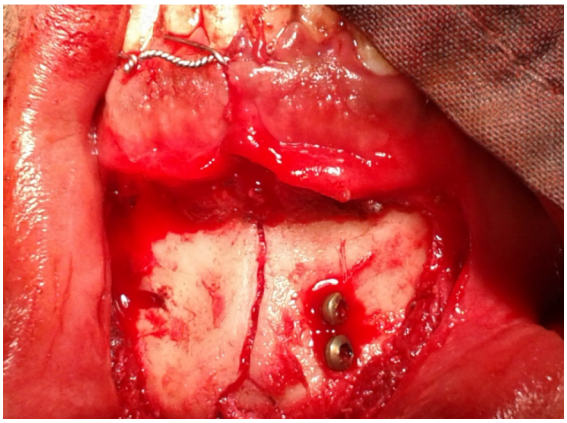

Figure 1 Symphyseal fracture fixed with two lag screws.

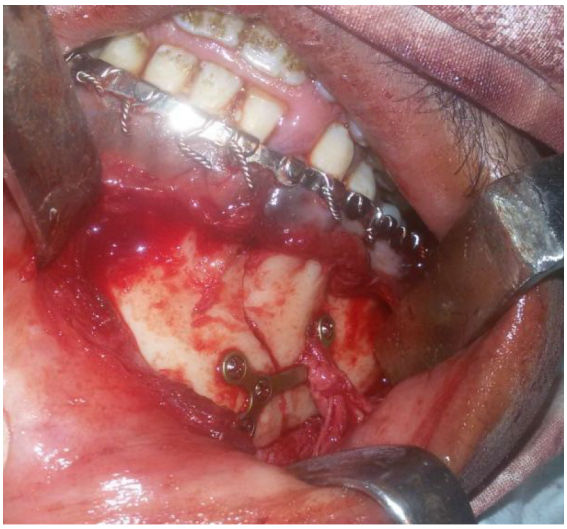

Figure 2 Parasymphyseal fracture fixed with double Y-shaped miniplate.

patients (18.8%). The fractures comprised 10 parasymphyseal fractures (62.5%) and 6 symphyseal fractures (37.5%).

In group 1, patients were treated with open reduction and internal fixation using lag screws and the mean operating time from start of hardware application to end of fixation was 14.38 ± 1.92 min. In group 2, patients were treated with open reduction and internal fixation using double Y-shaped miniplates and the mean operating time was 15.63 ± 1.53 min. The difference between the two groups regarding the mean operating time was statistically insignificant ($P > 0.05$) (Table 1).

With regard to postoperative edema, only 2 patients in the study sample showed severe edema (12.5%), while all other patients demonstrated mild to moderate edema (87.5%) on the second postoperative day. By the end of the first week, the edema has resolved completely in all patients.

The mean pain intensity in the first postoperative week was 4.125 ± 1.25 in group 1 and 4.75 ± 1.04 in group 2 with no statistically significant difference ($P = 0.294$). Pain was completely resolved by the end of the second week.

The mean maximal mouth opening measured two weeks after surgery was 38.25 ± 2.38 mm in group 1, and 37.63 ± 2.92 mm in group 2 with no statistically significant difference ($P = 0.646$).

The surgical wounds healed uneventfully in all patients in both groups except for one patient in group 2 who had wound dehiscence that was managed conservatively using irrigation and antiseptic mouth washes until secondary intention healing was achieved. No sensory nerve impairment was detected postoperatively in any of the patients in either group. Satisfactory occlusion and normal inter-cuspal relation were evident in all patients except for one patient in group 1 who had slight malocclusion postoperatively, that was managed by selective grinding.

Table 1 Mean operating time

	Group	*n*	mean ± SD	*t*	Significance (2-tailed)
Operating time	1	8	14.3750 ± 1.92261 min		0.172
Operating time	2	8	15.6250 ± 1.52947 min	-1.439	

The mean bone density at the fracture line [measured in grey scale using the CBCT OnDemand3D™ software (310 Goddard Way, Suite 250 Irvine, CA, United States, https://www.ondemand3d.com)] at 3 mo postoperatively was 946.38 ± 66.29 in group 1 and 830.36 ± 95.53 in group 2. The difference between the two groups was statistically significant (P = 0.015). At 6 mo postoperatively, the mean bone density in group 1 was 1062.66 ± 63.89 and in group 2, it was 1083.86 ± 82.83, with no statistically significant difference between the 2 groups (Table 2).

DISCUSSION

The current study compared the use of lag screws *vs* double Y-shaped miniplates in the fixation of anterior mandibular fractures and comparable results were found in most evaluated parameters except for a statistically significant higher mean bone density in the lag screw group at 3 mo postoperatively.

The male to female ratio in the study sample showed a marked male predilection (4.33: 1) in agreement with other studies[1,6]. It is suggested that high-speed driving and greater participation in outdoor activities are probably more characteristic in men rather than women in our society, which renders them more susceptible to accidents in that age group. Moreover, in accordance with previous studies, road traffic accidents were the major cause of trauma followed by personal violence and other causes[1,7].

The present study demonstrated a comparable mean operating time in both groups with no statistically significant difference, starting from hardware application to the end of fixation. This is in contrast to other studies which have shown shorter time for lag screw fixation in comparison to miniplates[8,9].

Mean pain score at the end of the first week was numerically (but not statistically) lower in the first group. Bhatnagar *et al*[10] obtained similar results with less pain in the lag screw group, and they explained their findings by the higher stability of the fracture line provided by lag screws in comparison to miniplates and less hardware applied leading to reduced persistent postoperative pain.

No postoperative sensory nerve impairment was detected in either group after fracture fixation, owing to the gentle fracture manipulation, careful dissection of the mental nerve and cautious application of screws in close proximity to the nerve. This is concordant with the results of the study by Agarwal *et al*[11] who did not observe any postoperative nerve deficit and stressed the importance of skills and patience during hardware application in anterior mandibular fractures.

The difference in mean bone density was statistically significant between the two groups at 3 mo post-operation suggestive of early bone healing. This is consistent with previous studies[9,12] using lag screws in fractures of the anterior mandible. This may be due to their compressive effect on the fracture segments, facilitating the progression of primary bone healing. However, by the end of the follow-up period, both groups had comparable mean bone density values indicative of adequate fracture healing and stability. Double Y-shaped miniplates with their special design have shown predictable biomechanical behavior with greater resistance to displacement when compared with straight miniplates[5].

To our knowledge, this is the first clinical trial comparing lag screws to double Y-shaped miniplates in the fixation of anterior mandibular fractures. This special design of miniplates provides better stability than straight miniplates and easier application/adaptation than 3-dimensional miniplates in the anterior region. However, the main limitation of the current study is the small sample size, which in some way, might have affected interpretation of the results. The small number of patients included is attributed to the meticulous case selection to meet all the inclusion criteria and minimize the variability between cases as much as possible.

Table 2 Mean bone density in the 2 groups at 3 and 6 mo postoperatively

	Group	*n*	mean ± SD	*t*	Significance (2-tailed)
Bone density 3 mo post-operation	1	8	946.3825 ± 66.29304	2.822	0.015[a]
	2	8	830.3625 ± 95.52573		
Bone density 6 mo post-operation	1	8	1062.6575 ± 63.88916	-0.573	0.576
	2	8	1083.8550 ± 82.82562		

[a]$P < 0.05$.

CONCLUSION

Both lag screws and double Y-shaped miniplates provide favorable means of fixation for mandibular fractures in the anterior region. Fractures fixed with lag screws show greater mean bone density at 3 mo post-operation, indicative of higher primary stability and faster early bone healing. Further studies with larger sample sizes are required to verify these conclusions.

ARTICLE HIGHLIGHTS

Research background

Several methods of fixation are available for the management of anterior mandibular fractures.

Research motivation

It is important to find the most suitable method to provide optimal fixation and stability against torsional forces in these fractures.

Research objectives

The effectiveness of lag screws and double Y-shaped miniplates in the fixation of anterior mandibular fractures was compared.

Research methods

Sixteen patients divided into 2 equal groups were included in the study.

Research results

The values of all parameters were comparable between the 2 groups except for the mean bone density which was significantly higher in the lag screw group at 3 mo post-operation.

Research conclusions

Both methods provide favorable fixation for anterior mandibular fractures with lag screws apparently leading to higher primary stability and faster healing.

Research perspectives

Further studies to confirm this conclusion and to compare with other methods of fixation are recommended.

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SYSTEMATIC REVIEWS

Tocilizumab as treatment for COVID-19: A systematic review and meta-analysis

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Abstract

BACKGROUND

The majority of patients with coronavirus disease 2019 (COVID-19) have good prognoses, but some develop a critical illness that can lead to death. Evidence shows severe acute respiratory syndrome is closely related to the induced cytokine storm. Interleukin-6 is a key player; its role in systemic inflammation is well known.

AIM

To evaluate the effect of tocilizumab (TCZ), an interleukin-6 receptor antagonist, on the outcomes for patients with COVID-19 pneumonia.

METHODS

PubMed, EMBASE, SCOPUS, Web of Science, MedRxiv, Science Direct, and the Cochrane Library were searched from inception to 9th June 2020 for observational or prospective studies reporting results of hospitalized adult patients with COVID-19 infection treated with TCZ. Effect sizes were reported as odds ratios (ORs) with 95% confidence intervals (CIs), and an OR less than 1 was associated with a better outcome in those treated with TCZ.

RESULTS

Overall 13476 patients (33 studies; n = 3264 received TCZ) with COVID-19 pneumonia and various degree of severity were included. Outcome was improved with TCZ. In the primary analysis (n = 19 studies reporting data),

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mortality was reduced in patients treated with TCZ (OR = 0.64, 95%CI: 0.47-0.87; $P < 0.01$). In 9 studies where risk of death with TCZ use was controlled for other variables mortality was reduced by 57% (OR = 0.43, 95%CI: 0.27-0.7; $P < 0.01$). Intensive care need (mechanical ventilation) was also reduced (OR = 0.36, 95%CI: 0.14-0.89; $P = 0.02$).

CONCLUSION
In COVID-19-infected patients treated with TCZ, outcome may be improved compared to those not treated with TCZ.

Key Words: Tocilizumab; COVID-19; Pandemic; Treatment; Meta-analysis; Review

Core Tip: Coronavirus disease 2019 (COVID-19) infection is associated with a citokine storm during acute phase. Interleukin-6 is a key player in this systemic inflammation. We evaluated the effect of tocilizumab (TCZ) on the outcomes of COVID-19 pneumonia. Mortality was reduced in patients treated with TCZ (Odds ratio =0.64, 95% confidence intervals: 0.47-0.87; $P < 0.01$). We conclude that TCZ may improve outcome of COVID-19 infected patients.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 emerged in Wuhan, China in December 2019 and a pandemic was declared by the World Health Organization on March 11, 2020. The pandemic rapidly became a major global health concern. The vast majority of patients with coronavirus disease 2019 (COVID-19) have good prognoses, but some develop a critical illness that can lead to death. The data show that approximately 20% become severe or critical and require hospitalization[1]. Evidence shows that severe deterioration following severe acute respiratory syndrome coronavirus 2 infection is closely related to the associated cytokine storm[2]. Tocilizumab (TCZ) is an immunomodulatory therapeutic, an interleukin (IL)-6 receptor antagonist approved by the United States Food and Drug Administration and the European Medicine Agency for treating cytokine release syndrome. One of the key cytokines described in the cytokine storm induced by COVID-19 is IL-6, and its role in systemic inflammation is well known. Following an intriguing biological rationale, several institutions have proposed using TCZ off-label to treat COVID-19[3]. Thus far, randomized controlled trials have not been reported in the literature, but observational studies and case reports describe the compassionate use of TCZ. Results leave the efficacy of TCZ controversial. We performed a meta-analysis of the studies available to date.

MATERIALS AND METHODS

Literature search and selection criteria

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were followed for evaluating records identified during the literature search[4].

The search included MEDLINE, EMBASE, Scopus, the medRxiv preprint server, Science Direct, Web of Science, and the Cochrane Controlled Register of Trials for articles published up to June 9, 2020 describing trials or observational series about the efficacy of TCZ in patients with COVID-19 pneumonia. Search terms were tocilizumab and COVID-19. The inclusion criteria were: (1) Randomized or single-arm prospective

studies, observational or retrospective case series of patients with COVID-19 and treated with TCZ outside of clinical trials; (2) written in the English language; (3) reporting patient clinical characteristics; and (4) including at least 5 patients. Animal studies, case reports, editorials, commentaries, and clinical or pharmacological reviews were excluded. If multiple studies reported on the same population and met the inclusion criteria, the newest study was selected unless different endpoints or subgroup analyses were performed or updated.

Data extraction and endpoints

Two authors (Ghidini A, Petrelli F) determined article eligibility based on the abstracts. A third (Zaniboni A) independently read the articles, and agreement for trial inclusion was reached. Two authors (Petrelli F, Ghidini A) independently extracted data to a standard form constructed using Microsoft Word and compared results for agreement. Extracted data were author, publication year, number of participants treated, study design, patient group demographics and clinical characteristics (*e.g.*, median age, sex, country, comorbidities), median follow-up, laboratory and clinical parameters (symptoms) of participants, rate of admission to the intensive care unit (ICU) before and after TCZ use, associated drugs, imaging (baseline and improvements shown in imaging), number of cycles with TCZ and resulting adverse events, death rate, median hospitalization time, rate of discharge from the ICU and/or hospital, and hazard ratios for mortality or other events associated with TCZ use.

Eligible studies were critically appraised by two independent reviewers at the study level for methodological and reporting bias by adapting the ROBIN-I tool[5] for assessing risk of bias in selected observational studies. By definition, single-arm or observational trials have a high risk of bias due to the absence of a control group and randomization. Otherwise, the Nottingham-Ottawa-Scale was used as a quality check for retrospective studies.

Statistical analysis

The primary endpoints were mortality (%) and ventilatory improvement (defined as the proportion of participants relieved from ICU admission or from non-invasive ventilation defined at the time from initiation of the study treatment) among those treated with TCZ. The outcome data extracted for each study were analyzed using random-effects models and were reported as weighted measures of any event. Event rates reported in individual studies were aggregated into pooled rates. All other continuous variables were analyzed using descriptive statistics. We used the procedures of the comprehensive meta-analysis (CMA) software to calculate the effect size using dichotomous outcomes; and if these were not available either, we used other statistics (such as *t*-value or *P* value) to calculate the effect size. A random-effects meta-analysis of odds ratios (ORs) was used to aggregate efficacy outcomes reported across trials. A meta-analysis of adjusted ORs attained from multivariate analysis only was also provided.

Heterogeneity was assessed using the χ^2 test. Statistical significance and the magnitude of I^2 were considered. When I^2 was less than 50%, low to moderate heterogeneity was assigned; otherwise, substantial heterogeneity was assigned. A significance threshold of $P < 0.05$ was adopted. All analyses were performed using CMA software version 2.2 (Biostat).

We tested publication bias by inspecting the funnel plot on primary outcome measures and by Duval and Tweedie's trim and fill procedure yields an estimated effect size after publication bias has been taken into account (as implemented in CMA). We also conducted Egger's test of the intercept to quantify the bias captured by the funnel plot and to test whether it was significant.

RESULTS

Thirty-three studies met inclusion criteria among 604 retrieved (Figure 1). The demographic and clinical characteristics of included studies are reported in Tables 1-3 (references reported in Supplementary material). Overall 13476 patients (n = 3264 received TCZ) with COVID-19 pneumonia and various degree of severity were included. The median age was 62 years. Almost all received treatments consisting of antibiotics (*e.g.*, azithromycin), antivirals, steroids plus or minus hydroxychloroquine. Mortality was 22.4% [95% confidence intervals (CIs): 17.9%-26.8%]. Ventilatory status improved in 63.9% (95%CI: 50.4%-75.6%).

Table 1 Baseline characteristics of tocilizumab treated patients

Ref.	Country	Type of study	No. of pts	Median follow up (d)	Male/Female, %	Median age (yr)	CV Comorbities, %	Respiratory/diabetes %	Other/cancer, %	Other medications, %	Ventilatory status (Baseline to end of follow up, %)	ICU admission %/time to ICU admission (d)
Alattar *et al*[11], 2020	Quatar	Retrospective	25	14	92/8	58	12 HTN	-/48	CKD 16/4	HCQ (100), AZITRO (96), lopinavir/ritonavir (96), ribavirin (88), and INF 1-α2a (60)	56 (invasive)	100/1
Alberici *et al* [12], 2020	Italy	Retrospective	6	4	-	-	-	-/-	-/-	Steroids, antivirals, HCQ	33 (16 worsened)	-/-
Capra *et al*[13], 2020	Italy	Retrospective (with ctr arm[1])	82 (*n* = 62 TCZ)	9	73/27	63	63 HTN	-/16	-/-	HCQ (100), lopinavir/ritonavir (100)	35.2 (27% worsened)	4.8/-
Colaneri *et al* [14], 2020	Italy	Retrospective with prop. score	112 (*n* = 21 TCZ)	7	90/10	62.3	47.6 HTN	0/9.5	19/4.7	HCQ, AZITRO, steroids (100)	-	14/-
Hassoun *et al* [15], 2020	United States	Retrospective	9	-	66/33	60	55 HTN	11/11	66/-	HCQ, AZITRO (100) steroids (33), antibiotics (66)	-	89/-
Klopfenstein *et al*[16], 2020	France	Case control	45 (*n* = 20 TCZ)	-	-	76.8	55 HTN/70 CVS disease	20/25	-/35	HCQ or lopinavir/ritonavir + antibiotics ± steroids (100)	-	0/-
Luo *et al*[17], 2020	China	Retrospctive	15	-	80/20	73	66 HTN	-/26.6	-/-	Steroids (53)	6.6 (33.3% worsened)	-/-
Quartuccio *et al* [18], 2020	Italy	Retrospective (with ctr arm[1])	111 (*n* = 42 TCZ)	17.8	78.6/21.4	62.4	47.6 HTN	-/-	-/-	Antivirals (100), HCq (92.9) steroids (40); antibiotics (28.6)	65 (invasive)	57/-
Sciascia *et al* [19], 2020	Italy	Prospective	63	-	89/11	62.6	45	4.7/9.5	-	Lopinavir/ritonavir (71), darunavir/cobicistat (29)	95	7.9/-
Toniati *et al*[20], 2020	Italy	Prospective	100	10	88/12	62	62	9/17	11/6	HCQ, lopinavir/ritonavir or remdesivir, antibiotic, steroids	69 (*n* = 23 worsened)	43/-
Xu *et al*[21], 2019	China	Retrospective	21	-	86/14	56.8	57.2	9.6/23.8	CKD 4.8/-	Lopinavir/ritonavir, IFN-α, ribavirin, steroids (100)	100	-/-
Ramaswamy *et al*[22], 2020	United States	Case control	86 (*n* = 21 TCZ)	-	61.9/38.1	63.2	14.3 HTN/heart disease, AF or stroke 19.1	28.6/14.3	-/0	HCQ (81), AZITRO (23.8), steroids (42.9)	-	47.6/-
Rimland *et al* [23], 2020	United States	Retrospective	11	17	82/18	59	73 HTN/18 CVS	27/36	Renal or liver 18/9	HCQ (36), AZITRO (64)	54 (10% worsened)	73/-

Sanchez-Montalva *et al* [24], 2020	Spain	Prospective	82	-	63/37	59.1	39 HTN/6.1 heart failure/12.2 AF	23.5/19.5	Liver 1.2/-	HCQ (98.9), lopinavir/ritonavir (76.8), AZITRO (96.3), darunavir/cobicistat (25)	53 (52% worsened)	2.9/-
Wadud *et al* [25], 2020	United States	Case control	94 (*n* = 44 TCZ)	-	-/-	55.5	-	-/-	-/-	-	-	-
Campochiaro *et al*[26], 2020	Italy	Retrospective	65 (*n* = 32 TCZ)	28	91/9	64	37 HTN/12 CAD	3/12	CKD 9/6	HCQ, AZITRO, lopinavir/ritonavir (100)	91	0/-
Morena *et al* [27], 2020	Italy	Prospective	51	30	78.4/21.6	60	29.4 HTN/49 CVS disease	9.8/11.8	5.9/5.9	HCQ (98), antibiotics (76), lopinavir/ritonavir (82), remdesivir (42)	66.6 (33% worsened)	11.8/-
Kimmig *et al* [28], 2020	United States	Retrospective (with ctr arm)	60 (*n* = 28 TCZ)	-	46.8/53.2	63.8	53.6 HTN/43 other	35.7/14.3	14/14.3	-	-	-
Roumier *et al* [29], 2020	France	Compassionate use	59 (*n* = 30 TCZ)	8	80/20	50	20 HTN/13 CVS	13/23	33/-	HCQ (6.6), steroids (6.6)	-	23.3/-
Ip *et al*[30], 2020	United States	Retrospective	547 (*n* = 134 TCZ)	30	78/22	62	71.6 HTN and coronary arthery disease	15/35	15/9	HCQ + AZITRO (92), steroids (66)	-	100/-
Perrone *et al* [31], 2020	Italy	Phase 2 and expansion cohort	1221 (*n* = 708 TCZ[3])	30	82/18	61% > 60	68 heart disease or HTN	-/15	-/-	HCQ (75), anti-retroviral (65), antibiotics (50), steroids (28)	-	16 invasive ventilation/-
Perez-Tanoira *et al*[32], 2020	Spain	Cohort study	562 (*n* = 36 TCZ)	-	-/-	-	-	-/-	-/-	-	-	-
Somers *et al* [33], 2020	United States	Observational	154 (*n* = 78 TCZ)	47	68/32	55	85 HTN or heart failure	54/13	CKD 35/-	HCQ (26), steroids (29), remdesivir (3)	56 (18 and worsened)	100/41 < 24 h, 36 > 48 h
Heili-Frades *et al*[34], 2020	Spain	Cohort study	4712 (*n* = 366 TCZ)[2]	-	-/-	-	-	-/-	-/-	-	-	40.7/-
Issa *et al*[35], 2020	France	Retrospective	10	-	100/0	66	60 HTN	-/30	-/-	HCQ (100), steroids (30)	50	70/7 d
Garcia *et al*[36], 2020	Spain	Retrospective	171 (*n* = 77 TCZ)	-	58.8/51.2	61.5	61 HTN or heart disease	10.3/15.6	-/-	Antivirals (100, steroids (50)	90	10.3/-
Ayerbe *et al* [37], 2020	United Kingdom	Retrospective	2075 (*n* = 421	8	-/-	-	-	-/-	-/-	-	-	-/-

			TCZ)									
Borku Uysal *et al*[38], 2020	Turkey	Retrospective	12	22	50/50	65.8	58 HTN	16/58	CKD 8/16	HCQ and antivirals (100), AZITRO (50), antibiotics (58)	82	17/-
Fernandez-Cruz *et al*[39], 2020	Spain	Retrospective	463 (*n* = 189 TCZ)	-	-/-	-	-	-/-	-/-	Steroids (100), other not available	-	-/-
Garibaldi *et al* [40], 2020	United States	Cohort study	832 (*n* = 39 TCZ)	-	-/-	-	-	-/-	-/-	-	-	-/-
Martínez-Sanz *et al*[41], 2020	Spain	Cohort study	1229 (*n* = 260 TCZ)	-	73/27	65	17 HTN, 8 CAD, 2 heart failure	18/15	CKD 4/-	-	-	19/6 d
Petrak *et al*[42], 2020	United States	Retrospective	145	-	64/36	58.1	-	-	-	Corticosteroids (60), HCQ + AZITRO (98.6)	-	-/-
Rossi *et al*[43], 2020	France	Case control	246 (*n* = 106 TCZ)	28	66/34	64	60 HTN, 23.6 CVS	16/45	-/5.7	Antibiotics (100), HCQ (83), steroids (40), lopinavir/ritonavir (0.9)	-	-/-

[1]Control arm consisted in patients treated with hydroxycloroquine + lopinavir/ritonavir before tocilizumab availability.
[2]Hospitalized cohort only.
[3]Modified intent to treat analysis. CVS: Cardiovascular disease; CAD: Coronary arthery disease; AF: Atrial fibrillation; HTN: Hypertension; CKD: Chronic kidney disease; pts: Patients; HCQ: Hydroxycloroquine; AZITRO: Azitromycin; -: Not availble; TCZ: Tocilizumab; ICU: Intensive care unit.

Outcome was improved with TCZ. In the primary analysis (n = 19 studies reporting data), mortality was reduced in patients treated with TCZ (OR = 0.64, 95%CI: 0.47-0.87; P < 0.01; Figure 2). In 9 studies where risk of death with TCZ use was controlled for other variables mortality was reduced by 57% (OR = 0.43, 95%CI: 0.27-0.7; P < 0.01). Intensive care need (mechanical ventilation) was also reduced (OR = 0.36, 95%CI: 0.14-0.89; P = 0.02). In all cases, a random effect model was used.

Egger's test indicated a significant publication bias (P = 0.01). Duvall and Tweedie's trim and fill procedure indicated 4 missing studies (see the funnel plot with imputed studies in Supplementary material). The adjusted effect size (after imputation of the missing studies) was 0.84 (95%CI: 0.63-1.14).

DISCUSSION

A large part of the ongoing research into COVID-19 infection is concentrated on finding an immunomodulatory therapy to down-regulate the cytokine storm, usually combining it with antiviral agents[6]. In fact, IL-6 binds either with transmembrane IL-6 receptors or soluble IL-6 receptors, and the resulting complex can combine with the

Table 2 Laboratory and radiological characteristics of patients treated with tocilizumab

Ref.	Fever (baseline) °C/%	O_2 sat. %	Cough %	Dyspnea %	Leucocytes 10^9/L	Lymphocites/Neutrophil 10^9/L	PLT 10^9/L	Hb g/dL	LDH	Liver tests IU/L	CRP mg/L	PCT ng/L	D-dimer	IL6 ng/L	Imaging %
Alattar *et al*[11], 2020	38/92	-	84	72	6.0	0.9/5.0	208	-	-	46/30	95.2	0.38	-	-	Infiltrates and ground glass opacities 100
Alberici *et al*[12], 2020	-/-	-	-	-	-	-/-	-	-	-	-	-	-	-	-	-
Capra *et al*[13], 2020	38/-	-	-	-	-	-/-	-	-	-	-	123	0.6	-	-	Bilateral pulmonary opacities 100
Colaneri *et al*[14], 2020	-/-	-	-	-	-	0.6/8.4	303	-	445	38/72	21.3	0.24	-	-	Interstitial lung disease 100
Hassoun *et al*[15], 2020	-/-	-	-	-	-	-/-	-	-	-	-/-	-	-	-	-	-
Klopfenstein *et al*[16], 2020	-/-	90	-	-	-	0.67/-	-	-	-	-/-	158	-	-	-	≥ 50% lung involvement 60
Luo *et al*[17], 2020	-/-	-	-	-	-	-/-	-	-	-	-/-	96	-	-	71	-
Quartuccio *et al*[18], 2020	-/-	-	-	-	5540	0.68/4.5	157	-	625	-/-	79.05	-	835	63.5	-
Sciascia *et al*[19], 2020	< 38/39.7	-	-	-	-	-	-	-	-	-	-	-	-	-	Bilateral pulmonary infiltrates
Toniati *et al*[20], 2020	> 37.5/85	-	55	73	6	0.78	177	13.6	413	55/39	97	-	525	41	Ground glass opacities and consolidation, bilateral pulmonary infiltration
Xu *et al*[21], 2019	-/100	-	66.7	-	6.3	0.97	170	-	370	31/29	75	0.33	0.8	153	Ground glass opacities and focal consolidation, peripheral and subpleural
Ramaswamy *et al*[22], 2020	-/-	-	-	-	-	1.1/6.7	200	-	-	60/43.5	15.9	2.2	2900	371	-
Rimland *et al*[23], 2020	-/-	-	-	-	8.5	-/0.8	230	-	1203	51/35	197.3	-	343.5	30.65	-
Sanchez-Montalva *et al*[24], 2020	37.7/91.5	94	86.6	65.9	9.2	0.86/	199	13.3	446	53/41	17.98	-	295	74.8	-
Wadud *et al*[25], 2020	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Campochiaro *et al*[26], 2020	37.6/-	-	-	-	-	-/-	-	-	469	-/-	156	-	-	-	-

Morena *et al*[27], 2020	74.5/-	-	62.7	54.9	9.1	0.8/7.3	230	-	470	48/39	189	-	1706	116	Bilateral pulmonary opacities 100
Kimmig *et al*[28], 2020	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Roumier *et al*[29], 2020	-	-	-	-	-	-	-	-	-	-	189	-	3712	-	-
Ip *et al*[30], 2020	80	-	78	80	-	-/-	-	-	-	-/-	-	-		-	-
Perrone *et al*[31], 2020	-/-	-	-	-	-	-/-	-	-	-	-/-	30	-	-	-	-
Perez-Tanoira *et al* [32], 2020	-/-	-	-	-	-	-/-	-	-	-	-/-	-	-	-	-	-
Somers *et al*[33], 2020	-/-	-	-	-	12.1	0.9/-	-	-	627	50/76	185	-	2400	-	-
Heili-Frades *et al* [34], 2020	-/-	-	-	-	-	-/-	-	-	-	-/-	-	-	-	-	-
Issa *et al*[35], 2020	-/100	-	-	-	-	-/-	-	-	-	-/-	246	-	1354	-	Ground glass opacities
Garcia *et al*[36], 2020	-/98.7	-	83	43	-	0.87/-	-	-	-	-/-	97	-	918	-	-
Ayerbe *et al*[37], 2020	-/-	-	-	-	-	-/-	-	-	-	-/-	-	-	-	-	-
Borku Uysal *et al* [38], 2020	-/92	92	100	67	6.1	1.09/4.3	180	13.8	259	33/39	54	-	599	-	Ground glass opacities
Fernandez-Cruz *et al* [39], 2020	-/-	-	-	-	-	-/-	-	-	-	-/-	-	-	-	-	-
Garibaldi *et al*[40], 2020	-/-	-	-	-	-	-/-	-	-	-	-/-	-	-	-	-	-
Martínez-Sanz *et al* [41], 2020	36.8/-	91	-	-	-	0.89/5.4	-	-	669	-/32	113	-	809	70	-
Petrak *et al*[42], 2020	-	-	-	-	-	-	-	-	538	-	53.3	-	1.3	-	-
Rossi *et al*[43], 2020	37.5/-	94	-	-	-	1.128/-	-	-	-	-	168	-	-	-	-

-: Not available; PLT: Platelets; Hb: Hemoglobin; CRP: C reactive protein; PCT: Procalcitonin C; IL-6: Interleukin-6; sat: Saturation; LDH: Lactate dehydrogenase.

signal-transducing component gp130 to activate the inflammatory response. In an emergent situation where no approved drugs are available and supportive measures are available only for critically ill patients, any new promising agent merits attention. A meta-analysis has correlated IL-6 concentration with COVID-19 severity. Those with severe cases show a 2.9-fold higher concentration than those without complications[7].

Table 3 outcome of patients treated with tocilizumab therapy

Ref.	N° TCZ administered (median doses)	Death %	Dismissed %	Median hospitalization (d)	TCZ AEs %	Comparison with other medications or no TCZ	NOS Scale	ROBIN risk
Alattar *et al*[11], 2020	1	12	36 (from ICU)	-	Anemia 64; ALT ↑ 44	HR for discharge from ICU 0.64 (0.37-1.11)	8	Low
Alberici *et al* [12], 2020	1	33	16	-	-	-	6	Moderate
Capra *et al*[13], 2020	1	8	92	12.5	-	OR for OS 0.036 (0.07-0.18)°	7	Low
Colaneri *et al* [14], 2020	2	23.8	85.7 (from ICU)	2	0	OR for OS 0.78 (0.06-9.34); OR for ICU 0.11 (0-3.38)	7	Low
Hassoun *et al* [15], 2020	1	22	55	13.5 (n = 7)	-	-	5	Low
Klopfenstein *et al*[16], 2020	1 or 2	25	55	13	-	OR for OS and ICU admission 0.36 (0.1-1.3) and 0.03 (0.002-0.56); OR for mechanical vent 0.05 (0.003-0.93)	5	Low
Luo *et al*[17], 2020	1	20	-	-	-	-	5	High
Quartuccio *et al* [18], 2020	1	9.5	28.5	-	-	OR for OS 14.5 (0.76-278.3); OR for ICU admission 220.9 (12.7-3826.1)	8	Moderate
Sciascia *et al* [19], 2020	1 (2 in 82.5%)	11	-	-	-	-	6	Moderate
Toniati *et al*[20], 2020	1 (2 in 87%)	20	15	-	Septic shock (n = 2), GI perforation (n = 1)	-	8	Low
Xu *et al*[21], 2019	1 (2 in 14.3%)	0	100	15.1	-	-	5	Moderate
Ramaswamy *et al*[22], 2020	1 (2 in 38%)	14.3	-	-	-	HR for OS 0.25 (0.07-0.9)	5	Moderate
Rimland *et al* [23], 2020	1	27	18	18	-	-	7	Low
Sanchez-Montalva *et al* [24], 2020	1	26.8	41.5	-	-	-	6	Low
Wadud *et al* [25], 2020	-	38.6	-	-	-	OR for OS 0.58 (0.25-1.32)	6	Moderate
Campochiaro *et al*[26], 2020	1 (2 in 28%)	15	63	13.5	SAEs (25)	OR for OS 0.38 (0.11-1.27); OR for ICU admission 0.33 (0.13-8.5)	8	Low
Morena *et al* [27], 2020	-	27	61	-	AST/ALT ↑ 29, PLT 14, neutropenia 6, rash 2	-	8	Low
Kimmig *et al* [28], 2020	1 (2 in 10.7%)	42.9	25	-	Infections 71.4	OR for OS 2.25 (0.75-2.24)	6	Moderate
Roumier *et al* [29], 2020	1	10	20	-	-	OR for OS 0.25 (0.05-1.03); OR for ICU 0.17 (0.06-0.48)	7	Low
Ip *et al*[30], 2020	1 (78%)	46	-	-	Bacteriemia (13), secondary pneumonia (9)	OR for OS 0.66 (045-0.99)	8	Low
Perrone *et al* [31], 2020	1 (59.8), 2 (54.5)	20	-	-	26.4 G3-5; 14.4 G1-2	OR for 30-d OS 0.7 (0.41-1.22) and 1.22 (0.86-1.92) in phase 2 and validation cohort	8	Low
Perez-Tanoira *et*	-	27.7	-	-	-	OR for OS 1.015 (0.47-2.18)	5	Moderate

al[32], 2020								
Somers *et al* [33], 2020	1	18	56	20.4	Superinfection (54)	OR 0.39 (0.18-0.82)	8	Low
Heili-Frades *et al*[34], 2020	-	22.4	-	-	-	-	6	Moderate
Issa *et al*[35], 2020	1	10	-	11 (ICU)	-	-	5	High
Moreno-Garcia *et al*[36], 2020	-	10.3	84.4	-	-	OR for ICU 0.3 (0.12-0.71) and OR for OS 0.52 (0.21-1.29)	5	Moderate
Ayerbe *et al* [37], 2020	-	21.1	-	-	-	OR for OS 1.9 (1.44-2.51)	5	High
Borku Uysal *et al*[38], 2020	2	0	100	-	-	-	6	Moderate
Fernandez-Cruz *et al*[39], 2020	-	-	-	-	-	OR for OS 0.69 (0.41-1.19)	5	High
Garibaldi *et al* [40], 2020	-	5	-	-	-	OR for OS 1.14 (0.46-2.81)	5	Moderate
Martínez-Sanz *et al*[41], 2020	1	23	-	13	-	OR for OS 2.19 (1.54-3.1)	5	Low
Petrak *et al*[42], 2020	1 (84.8), 2 (15.2)	28.3	48.3	-	-	-	5	Moderate
Rossi *et al*[43], 2020	1	28.9	-	-	-	HR for OS 0.29 (0.17-0.49)	8	Low

-: Not availble; NOS: Nottingham-ottawa-scale; ROBIN: Risk of bias of non-randomized studies; ALT: Alanine aminotransferase.

Siltuximab, a chimeric monoclonal antibody acting and blocking IL-6, is being tested in the SISCO study, including patients with acute respiratory distress syndrome related to COVID-19 infection (NCT04322188). Preliminary data from 21 patients showed a reduction in the C-reactive protein levels in 16 patients, a clinical improvement in 33% and disease stabilization in 43% of cases[8].

In this pooled analysis of 31 studies including 2898 patients treated with TCZ, we found a strong trend toward improved survival with the use of TCZ (a significant reduction in acute mortality risk by 36%). Tocilizumab administration was also independently associated with a 57% reduced risk of death in multivariable analysis. Tocilizumab reduced also the risk of mechanical ventilation and ICU admission by 64%. Overall mortality rate was 22%.

The limitations of these data are related to the observational nature of the studies, primarily monocentric and non-controlled. The population treated with TCZ was negatively selected for the worst clinical and inflammatory conditions. Also, due to the non-randomized design of all studies, final results might have been biased, and the added value of TCZ might not have been formally proven. However, despite a likely imbalance among clinical and laboratory baseline variables between the 2 groups, the effect of TCZ on clinical outcomes appears sustained. We finally recognize that some papers reported in the primary analysis were pre-printed in MedRxiv archive and not still finally reviewed and published in full.

At this time, 45 trials are underway to explore the contribution of TCZ when added to the standard of care for COVID-19. Four are in phase 3 trials: the COVACTA study (NCT04320615), in which TCZ is compared with placebo, the NCT04361552 study in which the control arm is represented by best practices, the COV-AID study (NCT04330638), a six-arm study including anakinra and the association of anakinra + TCZ, and the RECOVERY study (NCT04381936), also a six-arm study, including hydroxychloroquine, lopinavir/ritonavir, and low doses of steroids.

Recently, the use of hydroxycloroquine or chloroquine with or without a macrolide was associated with decreased survival and increased rate of ventricular arrhythmias in COVID-19 hospitalized patients[9]. Despite this alarming concern, article and data purity were subsequently questioned and article retracted. Similarly, results of a separate study with data attained from a different database, showed that hydroxycloroquine failed to reduce infection risk in people exposed to patients with confirmed COVID-19. Results indicated that the incidence of new illness compatible with

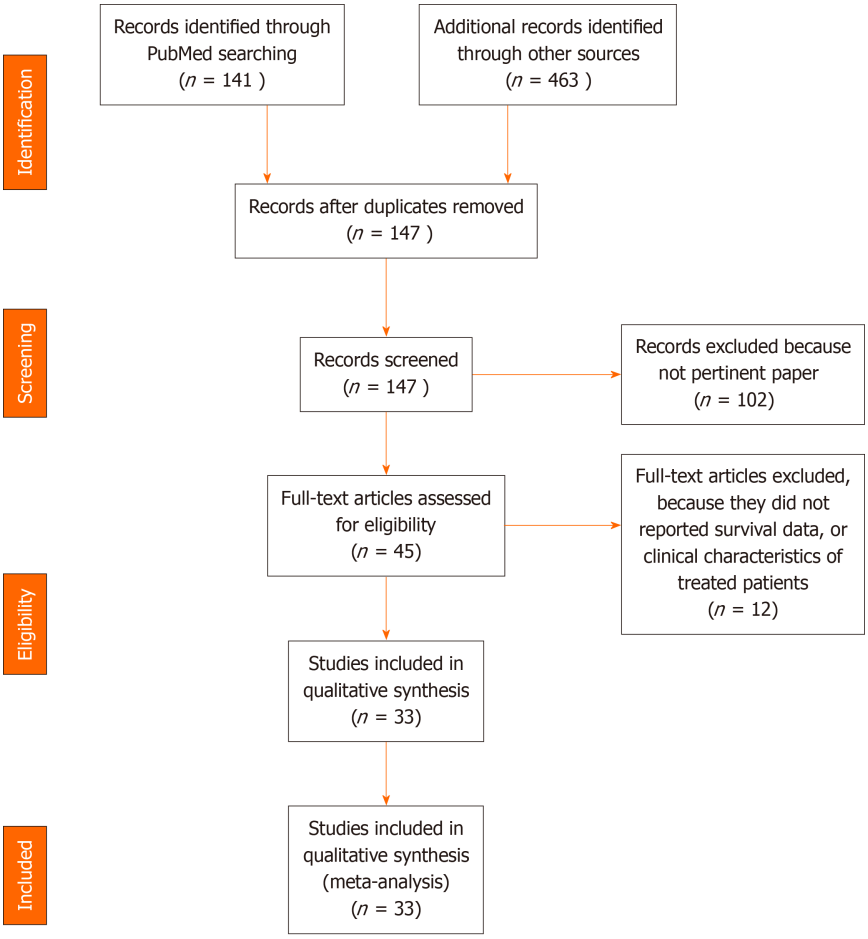

Figure 1 Thirty-three studies met inclusion criteria among 604 retrieved.

COVID-19 did not differ significantly between those who received hydroxycloroquine and those who received placebo[10]. Therefore, new combinations of potentially active drugs need to be tested, and efficacy confirmed in these patients[11-43].

CONCLUSION

In conclusion, we provide the first evidence that TCZ can improve the respiratory and clinical outcomes of patients with COVID-19 pneumonia in clinical practice, but its use merits further confirmatory trials.

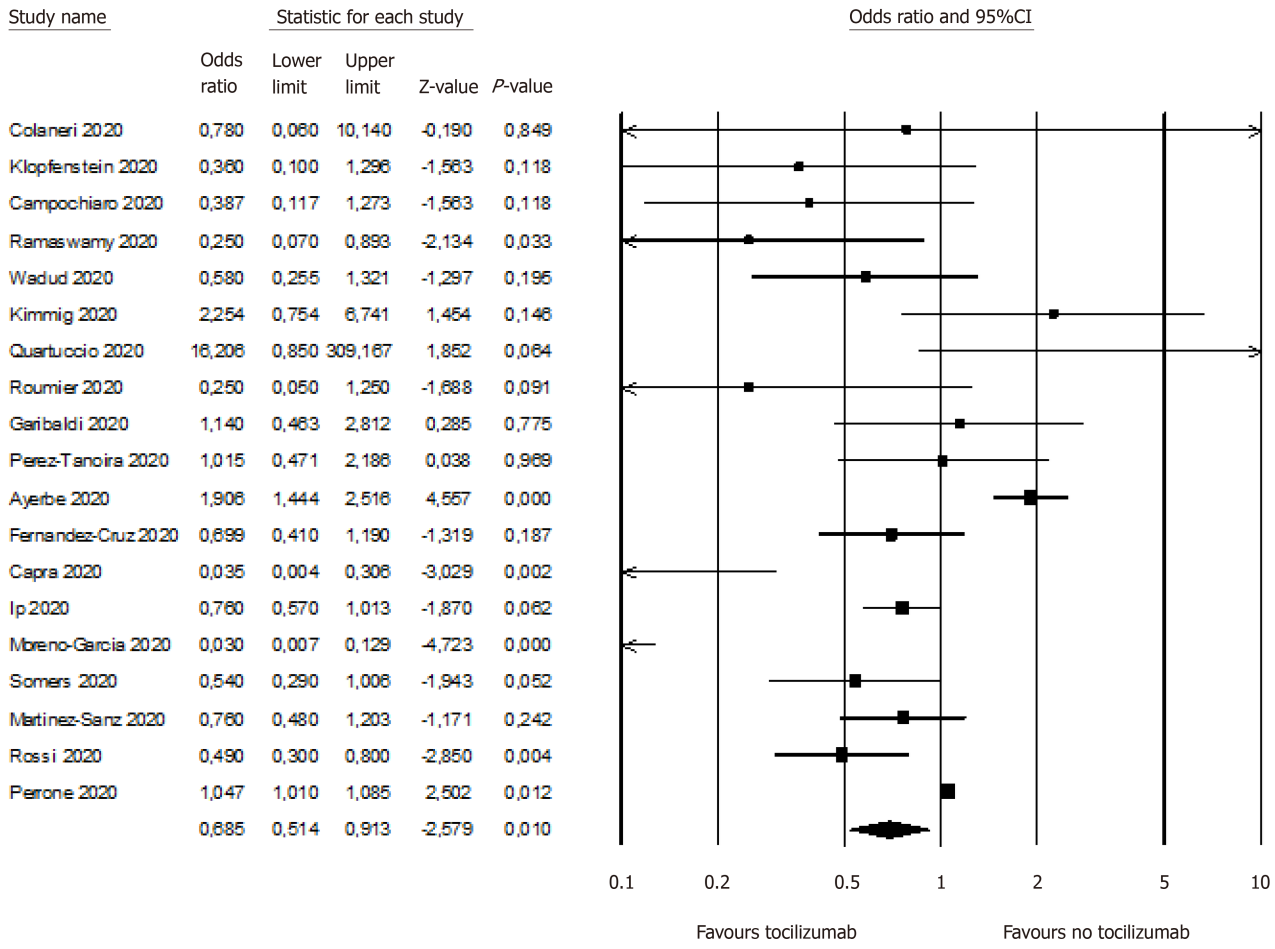

Figure 2 In the primary analysis, mortality was reduced in patients treated with tocilizumab.

ARTICLE HIGHLIGHTS

Research background

Coronavirus disease 2019 (COVID-19) infection is associated with a cytokine storm during acute phase.

Research motivation

Interleukin-6 is a key player in this systemic inflammation.

Research objectives

We evaluated the effect of tocilizumab (TCZ) on the outcomes of COVID-19 pneumonia.

Research methods

We performed a systematic review and pooled analysis of published literature.

Research results

Mortality was reduced in patients treated with TCZ (Odds ratio = 0.64, 95%CI: 0.47-0.87; $P < 0.01$).

Research conclusions

We conclude that TCZ may improve outcome of COVID-19 infected patients.

Research perspectives

Current use of tocilizumab in clinical practice has to be validated further through large randomized trials.

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