ISSN 2308-3840 (online)

World Journal of *Meta-Analysis*

World J Meta-Anal 2023 September 18; 11(6): 253-312

Published by Baishideng Publishing Group Inc

World Journal of Meta-Analysis

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Quarterly Volume 11 Number 6 September 18, 2023

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World Journal of Meta-Analysis

Quarterly Volume 11 Number 6 September 18, 2023

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INDEXING/ABSTRACTING

The *WJMA* is now abstracted and indexed in Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Meta-Analysis

ISSN
ISSN 2308-3840 (online)

LAUNCH DATE
May 26, 2013

FREQUENCY
Quarterly

EDITORS-IN-CHIEF
Saurabh Chandan, Jing Sun

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2308-3840/editorialboard.htm

PUBLICATION DATE
September 18, 2023

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World J Meta-Anal 2023 September 18; 11(6): 253-265

DOI: 10.13105/wjma.v11.i6.253

ISSN 2308-3840 (online)

REVIEW

Overview of angiogenesis and oxidative stress in cancer

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Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): E

P-Reviewer: Kocak A, Turkey; Raiter A, Israel; Shao A, China

Received: December 27, 2022
Peer-review started: December 27, 2022
First decision: January 30, 2023
Revised: March 7, 2023
Accepted: July 10, 2023
Article in press: July 10, 2023
Published online: September 18, 2023

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Abstract

Neoplasms can be considered as a group of aberrant cells that need more vascular supply to fulfill all their functions. Therefore, they promote angiogenesis through the same neovascularization pathway used physiologically. Angiogenesis is a process characterized by a heterogeneous distribution of oxygen caused by the tumor and oxidative stress; the latter being one of the most powerful stimuli of angiogenesis. As a result of altered tumor metabolism due to hypoxia, acidosis occurs. The angiogenic process and oxidative stress can be detected by measuring serum and tissue biomarkers. The study of the mechanisms underlying angiogenesis and oxidative stress could lead to the identification of new biomarkers, ameliorating the selection of patients with neoplasms and the prediction of their response to possible anti-tumor therapies. In particular, in the treatment of patients with similar clinical tumor phenotypes but different prognoses, the new biomarkers could be useful. Moreover, they may lead to a better understanding of the mechanisms underlying drug resistance. Experimental studies show that blocking the vascular supply results in antiproliferative activity *in vivo* in neuroendocrine tumor cells, which require a high vascular supply.

Key Words: Neuroendocrine lung tumors; Angiogenesis; Oxidative stress; Neuroendocrine serum markers; Neuroendocrine tissue markers; Future therapy

Core Tip: There are already several reviews in the literature that contribute to understanding angiogenesis and oxidative stress. However, this is the first review to report the latest cellular and molecular mechanisms of angiogenesis pathways while also discussing the genetics and biochemistry of oxidative stress in neoplasms. We also specifically discuss neuroendocrine lung tumors. These discoveries may be useful for new clinical and translational research studies.

Citation: Andriolo LG, Cammisotto V, Spagnoli A, Alunni Fegatelli D, Chicone M, Di Rienzo G, Dell'Anna V, Lobreglio G, Serio G, Pignatelli P. Overview of angiogenesis and oxidative stress in cancer. *World J Meta-Anal* 2023; 11(6): 253-265
URL: https://www.wjgnet.com/2308-3840/full/v11/i6/253.htm
DOI: https://dx.doi.org/10.13105/wjma.v11.i6.253

INTRODUCTION

The angiogenesis process consists of the generation of new blood vessels. The migration and proliferation of endothelial cells from already existing vessels to new vessels are crucial in this process. During embryonic development, these cells are particularly active, whereas in the adult their turnover is slow and limited to certain physiological phenomena, such as ovulation, tissue repair, and scarring processes[1].

Angiogenesis is the result of a well-balanced process between proangiogenic and antiangiogenic factors. This balance can fail due to specific stimuli such as hypoxia, creating a pathological angiogenic process[2]. The prevalence of proangiogenic factors is associated with serious diseases, such as cancer, and with inflammatory and degenerative diseases, such as retinopathies, rheumatoid arthritis, and psoriasis. Insufficient angiogenesis is the basis of obliterating vascular diseases, such as obstructive coronary artery disease or peripheral obstructive arterial disease (Buerger's disease), which are characterized by the downstream tissue ischemia of vascular occlusions[3].

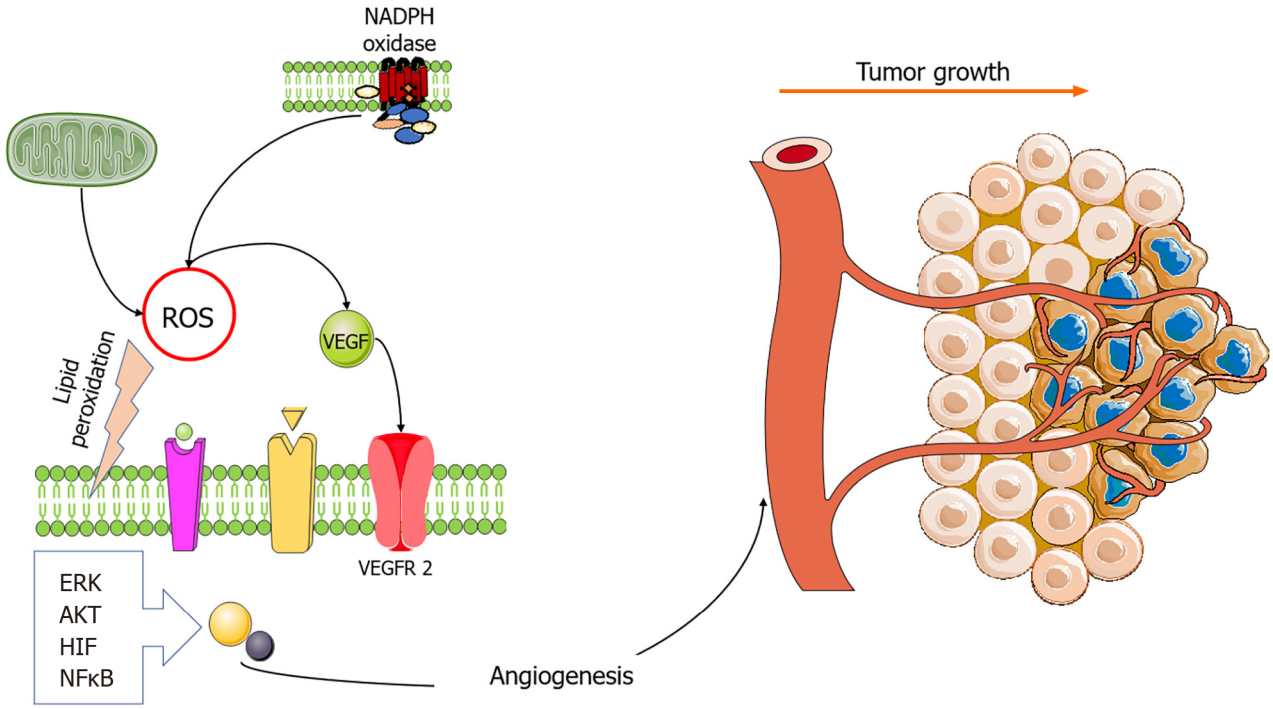
Neoplasms can be considered complex biological structures constituted by aberrant cells and endowed with specific functions; there are mesenchymal-derived cells, inflammatory cells, and vascular cells communicating with one another [4]. To fulfill all their functions, including growth and metastasis, they can promote angiogenesis through the same neovascularization pathway used physiologically. Tumor progression occurs due to the proliferation of the tumor cells themselves and the interactions that the neoplasm sets up within the tumor microenvironment where distinct types of tumor cells secrete key cytokines[5] for tumor progression and metastasis[6].

Cancer cells in active and continuous replication need a constant supply of oxygen and nutrients. For this reason, the first mechanism that cancer cells use to ensure the survival and growth of its cells is angiogenesis. However, neoplastic angiogenesis is an aberrant process associated with the formation of tortuous vessels that are insufficient to fulfill cellular needs. Acidosis is the consequence of altered tumor metabolism in response to hypoxia and the heterogeneous distribution of oxygen between the core and periphery that tumor angiogenesis helps to create. In this way, the acidic environment selects a more aggressive neoplastic cell phenotype with a greater invasive and metastatic phenotype.

Metabolic, hypoxic, and oxidative stress is considered a distinctive marker of cancer[7]. To survive the metabolic stresses, cancer cells activate different types of mechanisms including evasion of apoptosis and immune surveillance, increasing the angiogenic activity to enhance the provision of oxygen and nutrients, activation of the epithelial-mesenchymal transition (EMT), and metastasis[7,8]. Positive feedback between angiogenesis and oxidative stress is evident when a cellular mechanism stands for both the stimulus and the result of this process (Figure 1).

Tumor-induced angiogenesis begins with the release and activation of many growth factors[9]. The most important of which is vascular endothelial growth factor (VEGF) with its receptors. The mechanism of angiogenesis is complex, and it passes through stages well defined by changes in the endothelium and the extracellular matrix[10]. It can be schematically described as follows. The first stage of angiogenesis is characterized by the "destabilization" of pre-existing vessels and the loss of connection between endothelial cells due to increased vascular permeability. The proliferation phase of the endothelial cells follows with the formation of new vessels. Various proteolytic enzymes are released during these phases and alter the density of the extracellular matrix to help the migratory activity of endothelial cells. The third stage of angiogenesis is characterized by the formation of primitive capillaries. Finally, the last stage involves the recruitment of supportive periendothelial cells, such as pericytes and muscle cells, as well as the reorganization of periendothelial cells [11].

The most powerful stimulus for angiogenesis is hypoxia. Hypoxia and angiogenic factors released by the tumor destabilize the pericytes and stimulate continuous angiogenesis[12]. Tumors maintain hypoxia primarily due to the heterogeneous distribution of oxygen between the core and the periphery that cancer cells generate[13]; this situation is also associated with acidosis. By maintaining a low pH, cancer cells can evade immune cells and be chemoresistant[14].



DOI: 10.13105/wjma.v11.i6.253

Figure 1 The two main sources of oxidative stress, mitochondria, and nicotinamide adenine dinucleotide oxidases generate reactive oxygen species that trigger angiogenesis. The vascular endothelial growth factor (VEGF) pathway is modulated by reactive oxygen species (ROS), and oxidative stress stimulates VEGF production in several cell types, including endothelial cells. ROS enhance angiogenesis by increasing hypoxia-inducible factor (HIF) 1 α, protein kinase B (AKT), and regulated extracellular kinase (ERK). However, oxidative stress also induces angiogenesis in a VEGF-independent manner by lipid peroxidation and generating metabolites that act either as ligands or by inducing post-translational modifications of proteins within angiogenic signaling pathways, such as nuclear factor kappa-light-chain enhancer of activated B cells (NFκB) activation pathways. Figure was prepared using images from Servier Medical Art by Servier (https://smart.servier.com), which are licensed under a Creative Commons Attribution 3.0 Unsupported License. NADPH: Nicotinamide adenine dinucleotide; VEGFR2: Vascular endothelial growth factor receptor 2.

Reactive species, mainly represented by reactive oxygen species (ROS), are products generated by metabolic reactions that take place in the mitochondria of eukaryotic cells. If these reach a certain level they can be toxic to the cells. Physiological concentrations of reactive species can generally transduce signals before they are eliminated, whereas tumor cells need high concentrations of ROS to support their high proliferation rate due to their metabolism[15].

Among the several cellular strategies adopted by tumors to develop resistance to ROS are the so-called alternative metabolic pathways. These pathways prevent the accumulation of ROS without reducing the metabolic energy required by the tumor cells. The glycolysis with its parallel pathway and the pentose phosphate pathway, are examples of these pathways. The ROS levels are a sign of the damage that cells can withstand[16].

The therapeutic implications that follow are particularly important since the radiotherapy and chemotherapy currently available conduct their antitumor action precisely through the regulation of ROS levels. Therefore, the clinical response to pro-oxidant therapies has to be considered to enable truly personalized therapies. Consequently, the discovery of biomarkers capable of predicting this response is a challenge[17].

Somatostatin is a ubiquitous polypeptide produced by the delta cells of the digestive system and is present in the intramural plexuses of the intestine. Tumors originating from these cells produce and secrete somatostatin. Somatostatin exists in two biologically active forms, namely SS-14 and SS-28[18].

Several functions of somatostatin in the central nervous system are described. These include neuromodulatory, locomotor, and cognitive functions, inhibition of basal and stimulated secretion of distinct types of endocrine and exocrine cells, and regulation of cell proliferation and differentiation[19]. Specific membrane receptors are bound by somatostatin, of which there are five different subtypes called somatostatin receptors 1-5 (SSTR 1-5). These have maintained structural homology between distinct species (40%-60% of structural homologies) and mediate different biological actions by activating different intracellular signaling pathways[20,21].

Tumors that produce somatostatin have a typical histological architecture common to all neuroendocrine tumors (NETs) and a high somatostatin production. Somatostatin is a powerful inhibitor of neovascularization as many experimental data have shown. SSTR are expressed on endothelial cells, and the activation of quiescent endothelium is associated with an upregulation of SSTR2.

Somatostatin agonists inhibit VEGF, basic fibroblast growth factor, and growth hormone/insulin-like growth factor 1. Consequently, they can negatively regulate angiogenesis[22]. Furthermore, somatostatin can function as a powerful antitumor agent *in vivo* inhibiting both endothelial nitric oxide synthase and mitogen-activated protein kinases (MAPK) through SSTR3[23].

NETs represent a neoplasm that most benefit from metabolic radiotherapy and treatment with antiangiogenesis and pro-oxidant drugs. The presence of marked vascularization is a distinctive feature in most NETs, and this characteristic can be considered one of the diagnostic markers of neuroendocrine pathology[24]. Several studies have shown that microvascular density is 10 to 30 times greater in NETs than in other carcinomas[25].

TUMOR ANGIOGENESIS

As previously mentioned, the most important tumor-induced angiogenesis mediator is VEGF and its receptors[9] (Table 1). Six subtypes of VEGF are recognized: VEGF-A; VEGF-B; VEGF-C; VEGF-D; VEGF-E; and placental growth factor[26]. VEGF-C and VEGF-D take part in lymphangiogenesis. VEGF-A plays a dominant role in the angiogenesis process and is simply referred to as VEGF[27].

VEGF gene transcription is regulated by hypoxia-inducible factor (HIF), which is a protein composed of a constant subunit (HIF-1β) and an oxygen-regulated subunit (HIF-1α or HIF-2α)[28]. In response to hypoxia, the level of VEGF increases significantly in the extracellular space. High concentrations of VEGF determine the degradation of the basement membrane and the destabilization of the pericytes, the growth of endothelial cells, and the formation of new vessels[29]. This process is highly involved in tumor progression and when small tumors receive their nourishment by passive diffusion[30]. Those over 2 mm^2 undergo the formation of a hypoxic central core that stimulates the angiogenesis process [31]. This phase is called the "angiogenic switch" and is the release of many mediators of angiogenesis by the tumor cells in response to the reduced oxygen supply[32].

There are different mechanisms by which neoplasms stimulate angiogenesis[33]. The first and most important mechanism is germinal angiogenesis, which leads to the formation of new vessels from pre-existing capillaries and small venules. The endothelial cells undergo reactivation resulting in the formation of small shoots that grow and migrate into the adjacent connective tissue. Subsequently, an immature vessel is formed, stabilizing after the recruitment of pericytes and the reconstitution of the basement membrane. The new vessels are characterized by fenestrated endothelial cells, a discontinuous basement membrane, and rare pericytes. Consequently, the vascular network is permeable without efficient flow regulation and has an aberrant morphology with irregularly branched and tortuous vessels[34].

Another mechanism of tumor neovascularization is co-optation. In this case, the cancer cells grow along the normal vascular network. This mechanism is mainly observed in the brain, liver, and lung. It is particularly important in the early metastatic processes. Intussusception is the division of a pre-existing vessel into two new vessels and has been described in some aggressive tumors. Finally, in the vascular mimicry mechanism, a formation of vessels from the tumor cells themselves is observed. This process is seen in many aggressive tumors[35].

Pericytes are smooth muscle cells that stabilize the vessel walls and protect the normal vessels themselves from anticancer drugs, guaranteeing and promoting their target action. Hypoxia and angiogenic factors released by the tumor destabilize the pericytes and facilitate continued angiogenesis[8]. The reduction in their number leads to an increase in permeability and consequently the interstitial fluid pressure[36]. This leads to a further reduction in perfusion, the distribution of anticancer drugs, and acidosis[37]. Interstitial fluid pressure can be considered a marker of response to anticancer therapy[38].

Hypoxia can promote chemoresistance by increasing the ATP-binding cassette efflux pumps. Hypoxic cells are less proliferative than their normoxic counterpart and are therefore less subject to the chemotherapeutic cytotoxic effect[39]. Hypoxia also contributes to reducing the response to immunotherapy because it reduces immune activity[40]. An increase in HIF1 levels prevents the activation of CD8+ T-helper lymphocytes, suppresses the cytotoxic effect of natural killer cells, and increases the expression of immunosuppressive mediators such as inducible nitric oxide synthase and interleukin (IL)-10 by dendritic cells.

Different therapeutic strategies have been developed in an attempt to make hypoxia an advantage. Drugs activated by an enzymatic reduction in a hypoxic environment with the production of cytotoxic compounds have been tested without a real confirmation in terms of clinical utility[41]. Similarly, attempts were made to increase the oxygen transport capacity of the plasma using hyperbaric therapy[42].

In 1993, Kim *et al*[43] treated a mouse model of rhabdomyosarcoma, glioblastoma, and leiomyosarcoma with anti-VEGF monoclonal antibodies, obtaining tumor growth arrest. Given the ineffectiveness of these antibodies *in vitro* this pioneering study showed how blocking the action of angiogenesis mediators had a direct effect on tumor growth. However, the effect of these drugs was not constant[44]. There are differences in antitumor responses based on dosage, duration of treatment, and tumor type.

Due to the tremendous vascularization that characterizes them, neuroendocrine lung tumors would most benefit from antiangiogenesis drugs. This observation refers to the architecture of normal endocrine glands that need a well-represented vascular network that allows continuous exchange between endocrine cells and the bloodstream including hormone secretion.

Another characteristic of NETs that would suggest an elective use of antiangiogenic therapy as the treatment of choice is their marked ability to synthesize and secrete elevated levels of VEGF-A[45]. In this aspect, they mimic the endocrine cells with the secretion of peptide hormones[46]. Pancreatic islet β cells show the secretion of elevated levels of VEGF-A, which appears to play a significant role in the development of the dense vascular network of normal endocrine tissues [47]. VEGF-induced angiogenesis is also important for tumorigenesis and tumor progression of NETs. The angiogenic phenotype is necessary for the transition from hyperplasia[48], and it can be blocked pharmacologically[49]. Even in this process, VEGF-A plays a decisive role[50].

Table 1 Proliferation, migration, and differentiation by several factors/inductors implicated in angiogenesis

Inductors	Proliferation	Migration	Differentiation
Heparin-binding peptide growth factors			
VEGF	Yes	Yes	Yes
PlGF	Weak	Yes	Unknown
FGF-1, FGF-2	Yes	Yes	Yes
PTN	Yes	Unknown	Yes
HIV-tat protein	Weak	Weak	Yes
PDGF	Yes	Yes	Yes
HGF/SF	Yes	Yes	Yes
Peptide growth factors that do not bind heparin			
TGF-α	Yes	Yes	Yes
TGF-β	Inhibition	No	Yes
EGF	Yes	Yes	Yes
IGF-I	Yes	Yes	Yes
Inflammatory mediators			
TNF-α	Inhibition	No	Yes
IL-8	Yes	Yes	Unknown
IL-3	Yes	Yes	Yes
Prostaglandins E1, E2	No	No	Yes
Enzymes			
PD-ECGF/TP	No	Yes	Unknown
COX-2	No	Yes	Yes
Angiogenin	No	Yes	Yes
Hormones			
Estrogen	Yes	Yes	Yes
Proliferin	Unknown	Yes	Unknown
Oligosaccharides			
Hyaluronan oligosaccharides	Yes	Yes	Yes
Gangliosides	Unknown	Unknown	Unknown
Hematopoietic factors			
Erythropoietin	Yes	Unknown	Yes
G-CSF	Yes	Yes	Unknown
GM-CSF	Yes	Yes	Unknown
Cell adhesion molecules			
VCAM-1	No	Yes	Unknown
E-selectins	No	Yes	Yes
Integrins	No	Yes	Yes
Semaphorins (Sema3 e 4D)	No	Yes	Yes
Other			
Nitric oxide	Yes	Unknow	Unknow
Angiopoietin-1	No	Yes	Yes

COX-2: Cyclooxygenase 2; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; G-CSF: Granulocyte colony stimulating factor; GM-CSF: Granulocyte macrophage colony stimulating factor; HGF/SF: Hepatocyte growth factor/scatter factor; IGF: Insulin-like growth factor; IL: Interleukin; PD-ECGF/TP: Platelet-derived endothelial cell growth factor/thymidine phosphorylase; PlGF: Placental growth factor; PDGF: Platelet-derived growth factor; PTN, Pleiotrophin; TGF: Transforming growth factor; TNF: Tumor necrosis factor; VEGF: Vascular endothelial growth factor; VCAM: Vascular cell adhesion molecule.

The microvascular density of pancreatic NETs is higher in benign tumors than in malignant tumors and in this context is higher in low-grade than in high-grade malignant tumors. It is also characterized by a better prognosis. This observation is called the "neuroendocrine paradox." To explain this phenomenon, it has been hypothesized that in pancreatic NETs the vascular density is a marker of differentiation rather than of aggressiveness[51]. Like their normal counterpart, well-differentiated neuroendocrine cells do keep the ability to promote the formation of a dense vascular network. Conversely, the tumor angiogenesis mechanism of poorly differentiated neoplasms is secondary to hypoxia and aberrant genetic alterations. This does not signify the absence of angiogenic activity in well-differentiated NETs but that it is low per unit of time considered.

Little is known of the process of angiogenesis in NETs originating from organs other than the pancreas, and any available data are scarce and contradictory[52]. As far as the lung is concerned, it appears to be similar to the pancreas, with the presence of high vascular density in well-differentiated NETs and low in high-grade NETs. However, all aspects are not yet completely clear, and further studies are needed, particularly in the area of high-grade and metastatic cancers where antiangiogenic therapies would find their main application.

Several antiangiogenic target drugs have been successfully assessed in metastatic NETs such as anti-VEGFA, anti-VEGFR, and tyrosine kinase inhibitors. However, other drugs already in use in the therapy of NETs have also shown an antiangiogenic action. Among these are the analogues of somatostatin and interferon alpha. Somatostatin analogues have shown antiangiogenic properties *in vitro* by inhibiting the proliferation of endothelial cells and the synthesis and secretion of VEGF. However, data on their use *in vivo* are controversial, probably due to their insufficient ability to compete with VEGF and other proangiogenic factors[53]. The data in favor of the use of interferon alpha for the treatment of carcinoids seems more convincing. There is a significant reduction in intratumor microvascular density, but it is not associated with a reduction in circulating VEGF levels.

The development of resistance to antiangiogenic drugs is one of the major problems linked to their use, which is similar to other targeted therapies. This effect would explain the lack of long-term response and the so-called "angiogenic explosion" after their suspension. When anticancer drugs with antiangiogenic action are used at high dosages, they only have an acute antitumor effect that is not reflected long term.

Acute hypoxia due to massive and non-selective vascular destruction selects and facilitates only the most aggressive cancer cells, preventing immune surveillance, favoring metastases, and promoting resistance to anticancer treatments. Their use at low dosages as an adjuvant in chemotherapy regimens has instead shown efficacy thanks to the establishment of the so-called "vascular normalization" phenomenon[54]. This consists of the selective destruction of only immature and aberrant vascularity while respecting the normal one. Vascular normalization also passes through the fortification of the vessel wall as a result of the recruitment of pericytes. Finally, antiangiogenic drugs also determine a tumor microenvironment[40] effect of normalization due to the reprogramming of many tumor processes that target blood vessels.

Several studies showed[55] that the biological basis of resistance is not found in the genetic mutations that occur in the target molecules but rather in the establishment of a secondary angiogenesis pathway. Malignant cells can simultaneously synthesize and secrete many proangiogenesis factors, among which angiopoietin-2 seems to be the one that plays the most important role. This alternative route was observed in the experimental models of NET[56] and could justify both the increase in serum levels of angiogenic cytokines during anti-VEGF/VEGFR therapy and the simultaneous and effective use of combined therapies that block multiple angiogenic routes.

The use of angiogenesis markers could be a promising way to monitor the efficacy of antiangiogenesis therapy, determine its optimal dosage, avoid related toxicity, and predict its response or resistance. Currently, microvascular density is the best-known tissue biomarker. However, many data from the literature[57] show that it is not predictive in response to antineoplastic therapy. Different approaches have yet to be explored using immunohistochemical, molecular, and serum methods.

OXIDATIVE STRESS

Eukaryotic cells obtain the energy needed from aerobic respiration in the mitochondria. Due to this metabolic process, several reactive species are produced. They are required for signal transduction, enzymatic activity, gene expression, and protein folding in the endoplasmic reticulum and during apoptosis. Commonly, they are harmless. However, about 5% of reactive species can be toxic if they reach high concentrations.

Biochemistry of oxidative stress

The sources of oxidative stress can be both internal and external to the cell. Peroxisomes and P450 complex enzymes, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), xanthine oxidase, and NADPH complexes are all internal sources of oxidative stress. Almost all enzymes act within the mitochondria. Ultraviolet rays, chemicals (*e.g.*,

environmental pollutants, smoking, and alcohol), and exercise are, conversely, external sources of oxidative stress.

Based on the main atom involved we can divide the reactive species into four groups: ROS; reactive nitrogen species (RNS); reactive sulfur species; and reactive chloride species[58]. ROS and RNS are produced during the electron transport chain. ROS, which includes superoxide anion, hydrogen peroxide (H_2O_2), hydroxyl radical, singlet oxygen, and ozone, are the products of oxidative metabolism[59]. Some ROS, such as peroxynitrite anion and $ONOO^-$, can react with nitric oxide. Subsequently, nitric oxide is converted to a hydroxyl radical and a nitrite anion.

The balance between ROS and endogenous antioxidants determines the damage that cells can suffer. After the alteration of this balance, oxidative stress is generated with subsequent damage to DNA, RNA, lipids, and proteins[60]. Reactive species cause DNA damage and malfunctions in the DNA repair mechanisms. The oxidation of DNA that takes place generates 8-hydroxy-2-deoxyguanosine, which is a product capable of causing mutations in DNA and increasing cellular aging and carcinogenesis[61].

Polyunsaturated lipids are abundant in the cell membrane and are also particularly susceptible to oxidation by reactive species. By peroxidation reactions, they release lipids and increase the permeability of the cell membrane, which can lead to cell death[62]. However, proteins are the main target of the reactive species. The carbonyl (aldehydes and ketones) and thiol groups (–SH) can be converted into reactive sulfur radicals[63]. Therefore, there is an alteration in the structure of the protein that leads to changes or loss of function.

The cell has three groups of defense mechanisms: endogenous antioxidants; natural antioxidants; and synthetic antioxidants[64]. The following are endogenous antioxidants: glutathione; alpha-lipoic acid; coenzyme Q; ferritin; uric acid; bilirubin; metallothionein; l-carnitine; melatonin; superoxide dismutase; catalase; glutathione peroxidase; thioredoxin; and peroxiredoxin (PRX). PRX is a group of ubiquitous antioxidant enzymes (PRX I-VI). They can modulate the H_2O_2 levels and transduce intracellular signaling. PRX III eliminates up to 90% of H_2O_2, and PRX V is even more effective against peroxynitrite.

The diet is a source of natural antioxidants such as ascorbic acid (vitamin C), tocopherol (vitamin E), carotene (vitamin A), lipoic acid, uric acid, glutathione, and polyphenolic metabolites. Finally, synthetic antioxidants include N-acetyl cysteine, thyroid hormones, pyruvate, selenium, butylated hydroxytoluene, butylated hydroxyanisole, and propyl gallate [65].

Clinical importance of oxidative stress

Several human diseases, such as neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis), inflammatory diseases (arthritis), cardiovascular disease (atherosclerosis), allergies, immune system dysfunction, diabetes, aging, and cancer[66] are attributable to oxidative stress. During the acute inflammatory response, the chemical mediators released, such as ROS, also affect normal cells. In the case of a chronic inflammatory process, extremely high levels of ROS saturate the antioxidant mechanisms of the cell affecting the surrounding cells.

Oxidative stress in neoplasms

ROS are responsible for some cellular mechanisms implicated in tumor development and progression, including: (1) Cell proliferation (*e.g.*, activation of regulated extracellular kinase 1/2 and ligand-independent kinase receptor tyrosine kinase); (2) Apoptosis inhibition; (3) Tissue infiltration and metastasis (metalloproteinase secretion in the matrix extracellular, Met overexpression, and Rho-Rac interaction); and (4) Angiogenesis (release of VEGF and angiopoietin).

Several biochemical pathways are affected by oxidative stress (from epidermal growth factor receptor to mechanistic target of rapamycin) involving key signaling proteins, such as Nrf2, Keap1, Ras, Raf, MAPK, ERK1/2, MEK, p38, JNK, c-myc, p53, and PKC[67-69]. p38 acts as a key sensor of oxidative stress and is essential in the control of neoplastic development[70]. Unlike other MAPKs, p38 suppresses tumorigenesis by blocking proliferation and promoting apoptosis (Table 2).

Genetics of oxidative stress in neoplasms

A key role in the neoplastic transformation is played by genetic factors. A high level of ROS is associated with the increased metabolism observed in tumor cells; however, oxidative stress is less harmful to cancer cells than it is to normal cells. Cancer cells can adapt to the new conditions and proliferate, creating a new redox balance. This ability of cancer cells allows them to have a greater resistance to oxidation and oxidative stress than normal cells. It follows that the neoplastic cells can increase their metabolic rate and proliferation and avoid the damage caused by free radicals. However, this adaptive response alone cannot explain the high metabolic rate of tumors[71].

Genetic factors implicated in tumorigenesis may also directly or indirectly modulate ROS levels. The physiologic antioxidant activity is mainly regulated by the Nrf2 transcription factor in addition to specific antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, thioredoxin, and PRX. Nrf2 modulates the expression of many genes, including not only those that code for antioxidant enzymes but also genes that control immune and inflammatory responses, carcinogenesis, and metastasis[72]. ROS levels are controlled by Nrf2 and its repressor protein (Keap1). Furthermore, experimental data show that when treated with oxidation-inducing drugs Nrf2-free mice develop more severe intestinal inflammation than controls, suggesting a function for Nrf2 in preventing inflammation and carcinogenesis[73].

While Nrf2 was initially thought to be able to regulate oxidative stress by modulating the production of antioxidant enzyme antioxidant response element, subsequently kinase-dependent mechanisms have been described, such as MAPK, PI3K, and other alternative pathways for activation of Nrf2[74,75]. Somatic mutations that disrupt the Nrf2-Keap1 interaction have been identified in patients with non-small cell lung cancer[76] and esophageal cancer[77]. In breast cancer, the breast cancer tumor suppressor gene 1 (*BRCA1*) is mutated in 40%-50% of hereditary breast cancers, while it is absent or at a low level in 30%-40% of sporadic cases[78]. BRCA1 is responsible for DNA repair and can regulate Nrf2 and

Table 2 Molecular target of oxidative stress to promote tumor progression

Molecular target of ROS	Protein or gene	Function and mechanism	Tumor type
ERK1/ERK2	Protein	Promotion of cell proliferation and angiogenesis	Ovarian, colon, breast, and lung cancer
Nrf2	Protein	Regulation of oxidative stress by modulating the production of antioxidant enzymes	NSCLC and esophageal cancer
Ref1 /APE1	Protein	Reduction of ROS generation	Breast cancer
PTEN	Protein	Involvement in senescence; Association with high levels of Akt and ROS	Lung, liver, and breast cancer
Ras	Protein	Increases mitochondrial mass and ROS levels, causing DNA damage; Regulation of Nox4-p22phox system	30% of human cancer
mTOR	Protein	Promotion of cell proliferation and metabolism that contributes to tumor initiation and progression; Regulation of autophagy and apoptosis	More than 70% of cancers (breast, lung, colorectal, prostate, head and neck, gynecologic, urinary bladder, renal cancer gastric carcinoma, glioblastoma, lymphoma, and medullo-blastoma)
P38	Protein	Regulation of cell proliferation, cell differentiation, cell death, cell migration, and invasion.	Prostate, breast, bladder, live, and lung cancer, transformed follicular lymphoma and leukemia
BRCA	Gene	Regulation of antioxidant response; Controlling the Nrf2 and NFκB activity	40%-50% of hereditary breast cancers
hTERT	Protein	Reduces oxidative stress intracellularly and extracel-lularly; Regulation of apoptosis	Gastric cancer, lung cancer, cervical and head cancer, glioblastoma, breast cancer, and ovarian cancer
Angiopoietin	Protein	Involvement in angiogenesis, lymphangiogenesis, and metastasis; Induction of hypoxia and cytokines	NSCLC

Akt: Protein kinase B; *BRCA*: Breast cancer gene; ERK1/2: Extracellular kinase 1/2; hTERT: Human telomerase reverse transcriptase; NRF2: Nuclear factor erythroid 2-related factor 2; NSCLC: Non-small cell lung carcinoma; PTEN: Phosphatase and homolog of tensin; mTOR: Mammalian target of rapamycin; Ref/APE1: Redox factor/Apurinic/apyrimidinic endonuclease 1; ROS: Reactive oxygen species.

NFκB[79,80]. Nrf2 induces enzymes such as glutathione S-transferase, glutathione peroxidase, and oxidoreductase, which exert a protective action against ROS. In breast cancer cells the *BRCA1* gene reduces RNS damage to cells and helps them cope with oxidative stress. Redox factor 1/AP endonuclease 1 also participates in the reduction of ROS generation[81].

The Ras pathway (Ha-, N- and Ki-ras) is very important for regulating oxidative stress in cancer[82]. Ras activating point mutations are present in tumor cells (approximately 30% of tumors), resulting in a constitutively active protein. These mutations lead to an increase in ROS levels, which induces neoplastic transformation[83]. The *Ras* Val12 mutant activates the NOX4-p22phox NADPH oxidase system, which produces H_2O_2. Consequently, the response to *Ras* Val12-induced DNA damage is impaired by the inhibition of NADPH oxidase. NADPH oxidase, NOX4, can be considered a critical mediator of *Ras* Val12-induced oncogenic DNA damage[84].

If the *Ras* oncogene is overexpressed, cells show an increase in mitochondrial mass and an accumulation of ROS. Among these, the ROS generated by the respiratory chain in the mitochondria and the NOX enzymes in the cytoplasm are particularly important. NOX proteins are oncogenic proteins, and mitochondrial dysfunction is associated with tumorigenesis[85].

Mitochondrial dysfunction leads to DNA damage, decreased ATP levels, and activation of AMPK. The presence of the *K-ras* Val12 mutant in normal epithelial cells leads to increased peroxide levels and increased DNA damage. Peroxides can be generated by the COX-2 enzyme due to their correlation with K-ras[86]. Consequently, the COX-2 enzyme is also involved in many human cancers. Both peroxide production and DNA damage are reduced by pretreatment with the COX-2 antagonist SC58125. Therefore, several proteins including COX-2 and the transcription factor HIF-1α, which is activated in response to low oxygen concentrations, can influence the oncogenic activity of mutant K-rasVal12.

Overexpression of oncogenic proteins [Raf, reverse transcriptase of Mos, MEK, Myc, cyclin E and human telomerase reverse transcriptase (hTERT)] and inhibiting oncosuppressor genes (p53, p21CIP1, PTEN) can cause aging by increasing ROS levels. PTEN deficiency and Ras/MAPK activation could promote metastasis and EMT from prostate precursor cells [87]. Even in glioblastoma cells, PTEN deficiency, associated with high levels of Akt and ROS, leads to senescence. There is evidence that suggests the *hTERT* oncogene acts by modulating the redox system[88]. hTERT is localized in mitochondria, and its activity could influence the redox balance through the recruitment of the same mitochondria. Finally, hTERT inhibitors can induce mitochondrial-dependent apoptosis in target cells[89].

Many other genes are involved in regulating energy metabolism in cancer. *p53*, for instance, is one of the best-known tumor suppressors, and it is implicated in cellular energy balance in the mitochondria between glycolysis and the respiratory chain. Homologous cytochrome oxidase 2 is an important enzyme that mediates this effect, and its activity is very important for the regulation of the COX complex. Reduced homologous cytochrome oxidase 2 synthesis can cause low respiration and a high rate of glycolysis[90].

Sirtuins are a group of proteins involved in many cellular processes (aging, stress response, *etc*). Sirtuins are deacetylase enzymes regulated by NAD (positive activity) and NADH (negative activity). Sirt3 is the most studied of the three mitochondrial sirtuins and is known to act as a tumor suppressor. It is for this reason that it has been linked to longevity in humans. Kim *et al*[91] showed that in Sirt3 (-/-) murine embryonic fibroblasts, increased glycolysis, decreased oxidative phosphorylation, and increased ROS can be observed. Furthermore, the loss of Sirt3 increases cell tumorigenesis[92]. This process is accompanied by the activation of the HIF-1α target gene under hypoxic conditions.

NEUROENDOCRINE LUNG TUMORS

Bronchopulmonary neuroendocrine neoplasms represent a group of rare neoplasms (accounting for almost 20% of all lung neoplasms)[93] arising from the proliferation of cells with both endocrine and nervous phenotypic characteristics that together form the diffuse neuroendocrine system[94].

Based on their morphological, structural, immunohistochemical, and ultrastructural characteristics, they can be divided into four groups according to the 5th edition of the World Health Organization classification on thoracic tumors [95]: typical carcinoid (TC); atypical carcinoid (AC); large cell neuroendocrine (LCNEC); and small cell carcinoma (SCLC). TC and AC are considered well-differentiated NETs, while LCNEC and SCLC are considered poorly differentiated tumors. TC and AC are low (corresponding to G1 NET) and intermediate (corresponding to G2 NET) grades, respectively, whereas LCNEC and SCLC are high grades (traditionally graded as G3 tumors). Although these four subgroups of neuroendocrine neoplasms may represent a continuum in the neuroendocrine differentiation spectrum, histological, immunohistochemical, and molecular studies have demonstrated that pulmonary carcinoids are different from poorly differentiated neuroendocrine carcinomas[96].

The first description of a bronchopulmonary carcinoid dates back to 1831 when Laennec[97], in his treatise on mediated auscultation of the lungs and heart, reported the case of a posthumous endobronchial mass. The clinical presentation can occur with cough, hemoptysis, and recurrent pneumonia (due to the functional exclusion of a bronchus by a growing mass) even if in most cases their clinical course is indolent[93].

The diagnosis is based on imaging methods, such as computed tomography and magnetic resonance imaging, bronchoscopy, bronchial biopsy or fine-needle aspiration biopsy, mediastinoscopy (in selected cases), scintigraphy with 111 In-pentetreotide (octreoscan), and functional studies such as the evaluation of the tumor secretion pattern. Although less than 5% of patients with bronchopulmonary carcinoids have symptoms such as carcinoid syndrome, Cushing's disease, acromegaly, or syndrome of inappropriate antidiuretic hormone secretion, it is possible to detect secretion of amines, peptides, or hormones (endocrine, autocrine, or paracrine)[93].

However, the NETs most striking phenotypical characteristic is the massive vascularization[52] due to their marked ability to synthesize and secrete high levels of VEGF[45]. The experimental data available refer especially to the pancreatic NETs where the presence of high vascular density in NETs and low vascular density in neuroendocrine carcinoma is observed. The precise situation and the angiogenesis mechanism is not completely clear in neuroendocrine lung tumors. This review could provide a starting point for further future studies.

Experimental evidence has shown that the ROS released by the tumor due to metabolic stress are associated with different outcomes depending on their level[31]. Evidence shows that high levels of ROS directly lead cancer cells to cell death whereas low to medium ROS levels increase neoplastic progression, metabolism alteration, cell migration, EMT, and metastasis[98,99]. ROS also stimulate acute inflammation that becomes chronic when associated with prolonged ROS production[100]. NFκB and TGF-β are implicated in the relationships between chronic inflammation and carcinogenesis [101]. ROS are also responsible for p38 MAPK activation and TGF-β1-mediated EMT in many tumors[14]. Mitochondria are very important in determining neoplastic degeneration due to their production of endogenous ROS that subvert the metabolic process and oxidative phosphorylation[102].

Oxidative stress induces the production of ROS-dependent cytokines such as TGF-β, IL-6, IL-13, and VEGFA. A change to the mitochondrial redox and consequently the acid-base balance of the tumor microenvironment could represent a therapeutic strategy to improve the cellular function of T lymphocytes during immunotherapy treatment[103].

CONCLUSION

The use of angiogenesis and oxidative stress markers could be useful for evaluating the efficacy of antineoplastic drugs, establishing the optimal dosage, escaping from the related toxicity, and predicting its response or resistance.

FOOTNOTES

Author contributions: Andriolo LG and Cammisotto V designed the research study; Andriolo LG and Di Rienzo G performed the research; Andriolo LG, Cammisotto V, Spagnoli A, and Alunni Fegatelli D analyzed the data and wrote the manuscript; All authors read and approved the final manuscript.

Conflict-of-interest statement: All the authors declare that they have no conflicts of interest.

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S-Editor: Liu JH
L-Editor: Filipodia
P-Editor: Yu HG

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World Journal of Meta-Analysis

Submit a Manuscript: https://www.f6publishing.com

World J Meta-Anal 2023 September 18; 11(6): 266-276

DOI: 10.13105/wjma.v11.i6.266

ISSN 2308-3840 (online)

REVIEW

History, origin, transmission, genome structure, replication, epidemiology, pathogenesis, clinical features, diagnosis, and treatment of COVID-19: A review

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Specialty type: Virology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D, D
Grade E (Poor): 0

P-Reviewer: Moreno-Galarraga L, Spain; Shen F, China; Sultana N, Bangladesh

Received: April 4, 2023
Peer-review started: April 4, 2023
First decision: May 15, 2023
Revised: July 15, 2023
Accepted: July 25, 2023
Article in press: July 25, 2023
Published online: September 18, 2023

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Abstract

In December, 2019, pneumonia triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) surfaced in Wuhan, China. An acute respiratory illness named coronavirus disease 2019 (COVID-19) is caused by a new coronavirus designated as SARS-CoV-2. COVID-19 has surfaced as a major pandemic in the 21st century as yet. The entire world has been affected by this virus. World Health Organization proclaimed COVID-19 pandemic as a public health emergency of international concern on January 30, 2020. SARS-CoV-2 shares the same genome as coronavirus seen in bats. Therefore, bats might be its natural host of this virus. It primarily disseminates by means of the respiratory passage. Evidence revealed human-to-human transmission. Fever, cough, tiredness, and gastrointestinal illness are the manifestations in COVID-19-infected persons. Senior citizens are more vulnerable to infections which can lead to dangerous consequences. Various treatment strategies including antiviral therapies are accessible for the handling of this disease. In this review, we organized the most recent findings on COVID-19 history, origin, transmission, genome structure, replication, epidemiology, pathogenesis, clinical features, diagnosis, and treatment strategies.

Key Words: COVID-19; SARS-CoV-2; Severe acute respiratory syndrome; World Health Organization; Pathogenesis

Core Tip: An acute respiratory illness (COVID-19) is caused by a new coronavirus designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 belongs to β-coronaviruses, and it shares the same genome as coronavirus seen in bats. It primarily disseminates by means of the respiratory passage. Much evidence revealed human-to-human transmission. Fever, cough, tiredness, and gastrointestinal illness are the manifestations in COVID-19-infected persons. Various antiviral therapies are accessible for the handling of COVID-19 disease.

Citation: Mokhria RK, Bhardwaj JK, Sanghi AK. History, origin, transmission, genome structure, replication, epidemiology, pathogenesis, clinical features, diagnosis, and treatment of COVID-19: A review. *World J Meta-Anal* 2023; 11(6): 266-276
URL: https://www.wjgnet.com/2308-3840/full/v11/i6/266.htm
DOI: https://dx.doi.org/10.13105/wjma.v11.i6.266

INTRODUCTION

In December 2019, in Wuhan (China) an outbreak of pneumonia symptomatized by fever, dry cough, fatigue, and occasional gastrointestinal symptoms was revealed. Most of these pneumonia patients were associated with the Huanan Seafood Market, Wuhan, China which deals in fish and various live animal species (poultry, bats, marmots, and snakes) [1].

By using reverse transcription polymerase chain reaction (RT-PCR), researchers determined the reason for the above symptoms and the rapid spread of cases being a novel coronavirus named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), causative agent of the Coronavirus Disease-2019 (COVID-19)[2-4].

On 30 January 2020, World Health Organization (WHO) stated the novel coronavirus outburst in Wuhan, China, a global crisis[5]. Later on WHO accepted that SARS-CoV-2 has the ability to spread worldwide[6,7]. On 11 March 2020, the WHO announced COVID-19, a pandemic[8]. In successive months, several thousand people in different provinces of China and cities were invaded by the unchecked spread out of this disease[9]. Later, this disease traveled to various countries i.e. Thailand, Japan, Republic of Korea, Vietnam, Germany, United States, Singapore, and India. On comparison COVID-19 cases have overtaken the infected cases and deaths from Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS) at this point of the disease outburst[10]. The early effect of COVID-19 was so dreadful that the various countries had to implement phases of lockdowns. All age groups including children and pregnant women were badly affected due to this infectious disease.

CoVs (Coronaviruses) relates to the order Nidovirales and they have the largest RNA genome[11]. CoVs pertain to Coronaviridae family. They are positive single-stranded RNA-enveloped viruses. Four genera of CoVs are Alpha-, Beta-, Gamma-, and Deltacoronavirus. Seven human coronaviruses (HCoVs) have been revealed till now and they belong to the Alpha- and Betacoronavirus genera. The Alphacoronavirus genus includes HCoVNL63 and HCoV-229E and Betacoronavirus genus includes HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV, and the novel SARS-CoV-2[12-17]. The alphacoronaviruses (HCoV-NL63 and HCoV-229E) and the betacoronaviruses (HCoV-OC43 and HCoV-HKU1) generally induce common colds, but severe lower respiratory tract infections can also appear, notably in the old age persons and kids[18]. HCoV-NL63 infection causes croup (laryngotracheitis)[19,20], and HCoV-OC43 infection causes severe lower respiratory tract infection among kids[21]. SARS-CoV and MERS-CoV are zoonotic viruses that cause severe respiratory syndrome[11].

This review summarizes the latest findings on the history, origin, transmission, genome structure, replication, epidemiology, pathogenesis, clinical features, diagnosis, and cure of COVID-19.

HISTORY OF THE CORONAVIRUS

Human coronaviruses (229E and OC43) was first diagnosed in late 1960 as a reason for the common cold and were observed safe for human beings[22,23]. In Guangdong province in China in 2002–2003, a disease outbreak resulted in which a new coronavirus (β genera) originated in bats and was crossed to human beings by intermediate host of Himalayan palm civet cats[24]. This virus named SARS-CoV had a fatality rate of 10%[14,25,26]. This virus had been quickly spreading worldwide, particularly in Asia[27].

Almost ten years after SARS in year 2012, another highly pathogenic CoV, MERS-CoV, appeared in Middle East countries[17]. MERS-CoV, was also of bat origin, with dromedary camels as the intermediate host, and intermediate host reservoir species were also observed in goats, sheep, and cows[28]. MERS-CoV affected approximately 2000 people with approximately 34% mortality rate[17].

Recently, in December 2019, the novel Coronavirus 2019 (nCoV) or SARS-CoV-2 surfaced in Huanan Seafood Market, Wuhan (China) which cause pneumonia epidemic of unknown cause[29].

EPIDEMIOLOGY: ORIGIN, RESERVOIRS, AND TRANSMISSION OF COVID-19

COVID-19 was thought to be originated in Wuhan (China). Environment specimens from the Huanan seafood market in Wuhan, China were examined positive, suggesting that the COVID-19 virus originated there[30]. According to several reports, Bat might be the likely pool of SARS-CoV-2[31,32]. Bats are the natural pool of a range of CoVs, including SARS-CoV-like and MERS-CoV-like viruses[33-35]. When the genome of COVID-19 and Bat CoV RaTG13 was compared and analyzed by virus genome sequencing and it revealed 96.2% genome sequence similarity with the Bat CoV RaTG13 genome[24]. It revealed that bat CoV and human SARS-CoV-2 might share the same ancestor[36]. It had > 70% resemblance with the SARS-CoV[37]. The SARS-CoV-2 emanated from bats and intermediate animals through which it reaches humans is unknown. Present suspects are pangolins and snakes[37]. Figure 1 shows the transmission cycle of SARS-CoV-2.

It seems that majority of early COVID-19 cases had a contact record with the seafood market, in Wuhan, China[24,38]. There is the possibility of human-to-human (Transmission *via* Aerosols, Nosocomial-Related Infections & Maternal Transmission) spread in people who did not have vulnerable to the seafood market of Wuhan, China[39]. It is also revealed that 31.3% of COVID-19 patients have traveled a short time ago to Wuhan and 72.3% of patients who are nonresidents of Wuhan, have contact with people of Wuhan[40]. Instances of COVID-19 in different provinces of China and in almost all countries of the world were recorded in people who were returning from Wuhan City, China[37]. COVID-19 cases were observed in countries outside China with no travel history to China indicating human-to-human transmission locally[41].

In India during the early period (from March 2020 onwards), there was an alarming rise in COVID-19 patients but now the recovery rate from this disease is much more and the situation is under control now.

GENOME STRUCTURE OF CORONAVIRUSES

SARS-CoV-2 belongs to beta-coronaviruses. Genome of SARS-CoV-2 is positive-sense single-stranded RNA [(+) ssRNA] with a 5'-cap, 3'-UTR poly(A) tail. The SARS-CoV-2 genome length is < 30 kb, having 14 open reading frames (ORFs) which encode non-structural proteins (NSPs), structural proteins *i.e.* spike (S), envelope (E), membrane/matrix (M) and nucleocapsid (N), and accessory proteins[42,43].

Coronavirus virions have a diameter of about 125 nm and are spherically shaped[44,45]. The genomes of coronaviruses encode five structural proteins: The spike (S), membrane (M), envelope (E) glycoproteins, hemagglutinin esterase (HE), and nucleocapsid (N) protein. All virions have all envelope protein and N protein, but only some beta coronaviruses possess the protein hemagglutinin esterase (HE)[46].

S glycoproteins

These proteins are located outside the virion and contribute to its usual shape. The homotrimers of the S proteins create the sun-like appearance that assigns coronaviruses their name[44,47,48]. Through their C-terminal transmembrane domains, S proteins attach to the virion membrane and also join with M proteins[49]. Virion attachment to particular surface receptors present in the host cell's plasma membrane is made possible by the N-terminus of the S proteins[50].

M glycoproteins

Three transmembrane domains are present in M glycoproteins. Glycosylation of M proteins occurs in the Golgi body[51-53]. Alteration in M protein is required to enter virion into the cell and for protein to become antigenic[54-56]. The M protein aids to regenerate new virions.

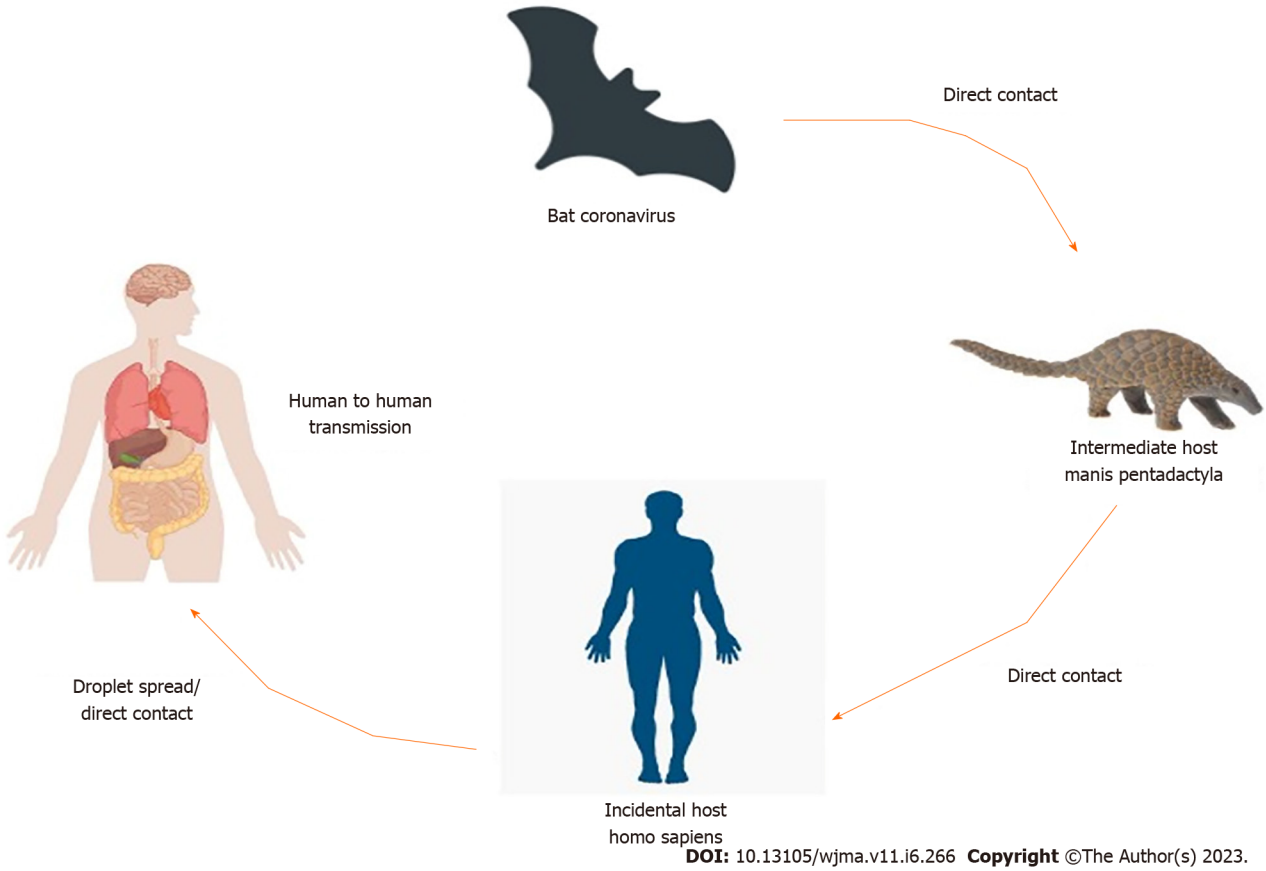
E glycoproteins

These are tiny proteins and are made from about 76 to 109 amino acids. The N-terminus of the E proteins typically has 30 amino acids, which facilitates adhesion to the virus membrane[57]. Additionally, Coronavirus E proteins perform an essential part in the assembly and morphogenesis of virions inside the cell.

N proteins

They are phosphoproteins in nature. They possess flexible viral genomic RNA and have the ability to bind to helixes. N proteins perform a vital part in coronavirus virion structure, replication, and transcription[58,59].

The complete genome of Wuhan-Hu-1 coronavirus, a strain of SARS-CoV-2 (taken from a COVID-19 pneumonia patient), is of 29.9 kb size[36]. The CoVs genome contains between 6 and 11 ORFs[60]. Two polyproteins named pp1a and pp1ab, encode 16 non-structural proteins, which are translated by approximately 66% of the viral RNA present in the first ORF (ORF1a/b). The remaining ORFs form structural and accessory proteins. Spike (S) glycoprotein, small envelope (E) protein, matrix (M) protein, and nucleocapsid (N) protein[11] are the four structural proteins encoded by the remaining part of the virus genome. SARS-CoV-2 is found to be more similar to SARS-like bat CoVs when compared with the known SARS-CoV and MERS-CoV genomes. The majority of genome-encoded proteins of SARS-CoV-2 are alike to those of SARS-CoVs. Zhang *et al*[61] observed that SARS-CoV-2 had been mutated in various patients in China. Tang *et al*[62] categorized two strains of SARS-CoV-2, the L type, and the S type. The L-type strains (derived from S-type) are more infectious and dangerous in terms of evolution than the S-type. As a result, virologists and epidemiologists must carefully examine the novel coronavirus and conduct additional research to determine its virulence and pandemic.



DOI: 10.13105/wjma.v11.i6.266

Figure 1 Transmission cycle of severe acute respiratory syndrome coronavirus 2.

CORONAVIRUS REPLICATION

Here, we summarise the main steps of the SARS-CoV-2 infection cycle.

Entrance into the host cell

The human lower respiratory tract has ACE2, the SARS-CoV receptor[63]. Coronavirus S-glycoprotein may bind to ACE2 receptor present on outer surface of human cells[64]. S glycoprotein comprises of S1 and S2 subunits[65]. S1 subunit specifies the virus-host range and cellular tropism with the help of the RBD domain, whereas S2 subunit helps the fusion of virus with cell membrane with the help of heptad repeats 1 (HR1)[66] and heptad repeats 2 (HR2)[67] domains.

RNA synthesis and virion assembly

After fusing with the membrane, genomic RNA of virus is delivered inside the cytoplasm. This RNA forms pp1a and pp1ab polyproteins after translation[68], which further form non-structural proteins, and replication-transcription complex (RTC) in two-layered vesicles[69]. RTC replicates repeatedly and forms a set of subgenomic RNAs[70], which further form accessory proteins and structural proteins. Newly generated genomic RNA, nucleocapsids, and envelope glycoproteins unite to form new viral particles in the ER and Golgi apparatus[71].

Virion release

At last, virion-containing vesicles combine along with the plasma membrane, and viruses are released outside.

EPIDEMIOLOGY AND PATHOGENESIS

This infection can affect people of any age. In humans, it is very contagious, especially in the elders and those who already have illnesses like fever, cold, or cough[72,73]. Large droplets released by symptomatic patients when coughing and sneezing are used to spread the infection; however, this can also happen from asymptomatic individuals prior to the start of symptoms[44]. COVID-19 infection transmits mainly by way of respiratory droplets, respiratory secretions, and direct contact[38]. Further, SARS-CoV-2 was also observed in faeces of severe pneumonia patients. Even after patients have recovered from the sickness, patients with symptoms can still spread infections. The infected droplets can deposit on surfaces and spread infection up to 1-2 meters away. In a suitable atmosphere, the virus can survive on surfaces for days. Disinfectants like hydrogen peroxide and sodium hypochlorite can destroy viruses[74]. Infection can be gained by

inhaling infectious droplets or by touching surfaces that have been exposed to the virus and subsequently contacting mouth, nose, and eyes. Further, virus is found in faeces and affects the water reservoirs and then spreads by faeco-oral route or through aerosolization[75]. Transplacental transfer from pregnant women to their foetuses has not yet been documented. Although, post-natal transmission in neonates is reported[76]. The incubation period of this virus ranges from 2 to 14 d[77].

CLINICAL FEATURES

The clinical characteristics of patients with COVID-19 are shown in Figure 2. Asymptomatic state, acute respiratory distress syndrome, and multi-organ failure are all possible clinical manifestations of COVID-19[37]. Fever, coughing, sore throat, headaches, sputum production, sore throat, lethargy, myalgia, shortness of breath, and conjunctivitis are frequent clinical symptoms[37]. Acute respiratory distress syndrome (ARDS), arrhythmia, shock[78], acute renal injury, acute cardiac injury, liver dysfunction, and secondary infection were the disorders related to this infection[40]. This infection can lead to pneumonia, respiratory failure, and even death after the first week. IL2, IL7, IL10, GCSF, IP10, MCP1, MIP1A, and TNFα are inflammatory cytokines that have dramatically increased during the advancement of this disease[79]. Recovery from this infection began in the second or third week. Elderly persons are more likely to experience negative effects which can lead to death[37]. Additionally, it has been noted that this disease in neonates, kids, and children is substantially less severe than in adults[37].

DIAGNOSTIC CRITERIA

An individual having fever, sore throat, and cough who has a traveling record to China or different locations with chronic community transmittance, or has contacted individuals having the same traveling experiences, or who have come into contact with a confirmed COVID-19 infected person is considered a suspected COVID-19 case[72]. A confirmed COVID-19 case is a suspected one having a positive molecular diagnostic test[72].

Until recently, the standard clinical diagnosis approach for COVID-19 is nucleic acid identification in swabs taken from nose, throat, or other parts of the respiratory system by using real-time polymerase chain reaction and furthermore verified by sequencing[80].

TREATMENT STRATEGIES FOR COVID-19

General precautions

COVID-19 patients are adequately isolated to stop infection to other persons in contact, patients, and health personnel. Keeping adequate water in the body and a proper diet plan while managing fever and cough are the best ways to treat moderate infection at home. It is advised to provide oxygen to hypoxic patients using nasal prongs, face masks, a high-flow nasal cannula, or non-invasive ventilation[72].

Four classes of medicines have been identified based on how they work: (1) Viral entry and membrane fusion inhibitors; (2) protease inhibitors; (3) RdRp inhibitors; and (4) immunomodulatory medicines.

Table 1 shows various therapeutic agents used for the treatment of COVID-19.

Umifenovir, camostat mesylate, ACE inhibitors, angiotensin receptor-1 blockers, soluble recombinant human ACE2, chloroquine phosphate, and hydroxychloroquine sulfate are the various medications that were tested to prevent attachment and fusion of the virus to the cell membrane[81]. Due to their increased production capacity and lower danger of antibody-dependent enhancement, MAbs act more efficient than convalescent plasma as medication for COVID-19 patients[82]. A new MAb cocktail called REGN-COV2 binds to the receptor-binding domain of S1 or S2 subunits of the SARS-CoV-2 spike protein to stop the virus from entering the host cell[83]. Three more MAbs (B38, H4, and CR3022), might be potent against SARS-CoV-2 in upcoming studies[84,85].

Another class of medications that have been used for a long time to treat AIDS is protease inhibitors. Under the trade name Kaletra®, lopinavir is commonly compounded with ritonavir (LPV/r). The LPV/r effectiveness has been demonstrated earlier in cell culture as opposed to SARS-CoV-1 and MERS-CoV[86] and in recent times opposed to SARS-CoV-2[87].

RdRp inhibitors, in particular, demonstrated encouraging results in COVID-19 patients[88-90]. For instance, Remdesivir (RDV, GS-5734, Gilead) inhibited the spread of SARS-CoV-2 at smaller doses[89]. Another RdRp inhibitor, favipiravir (T-705, Avigan®), has demonstrated efficacy against SARS-CoV-2 in Vero E6 cells at higher concentrations[89]. Another RdRp inhibitors, such as β-D-N4-hydroxycytidine (EIDD-1931), were very effective at stopping SARS-CoV-1, SARS-CoV-2, and MERS-CoV replication in *in vitro* condition[91].

To lessen the intensity and complexities of COVID-19 and escape the inflammatory immune reactions (in serious patients), a variety of therapy is frequently applied[92]. Proinflammatory cytokine-suppressing medications, including MAbs (tocilizumab and sarilumab) and IL receptor inhibitors (anakinra), are now available[93]. In Vero E6 cells, nitazoxanide showed antiviral activity as opposed to SARS-CoV-2[89].

Table 1 Various therapeutic agents used for the treatment of coronavirus disease 2019

Sr. No.	Therapeutic agents	Examples
1	Antiviral agents	1 Remdesivir
		2 Favipiravir
		3 Ribavirin
		4 Interferons
		5 Ritonavir/Lopinavir
		6 Arbidol
		7 Chloroquine/Hydroxychloroquine
		8 Recombinant soluble ACE2
		9 Azithromycin
		10 Ivermectin
		11 Nitazoxanide
		12 Camostat mesylate
		13 Paxlovid
2	Biologic agents	1 Monoclonal antibodies
		2 Convalescent plasma
		3 Hyperimmune sera
		4 Exogenous surfactant delivery
3	Anti-inflammatory agents	1 Corticosteroids
		2 Fluvoxamine
		3 Anakinra
		4 Granulocyte-macrophage colony-stimulating factor inhibitors
		5 Intravenous immunoglobulin
		6 Janus kinase inhibitors
		7 Colchicine
4	Herbal agents	Various Chinese herbal medicine
5	Preventive agents	Vaccines

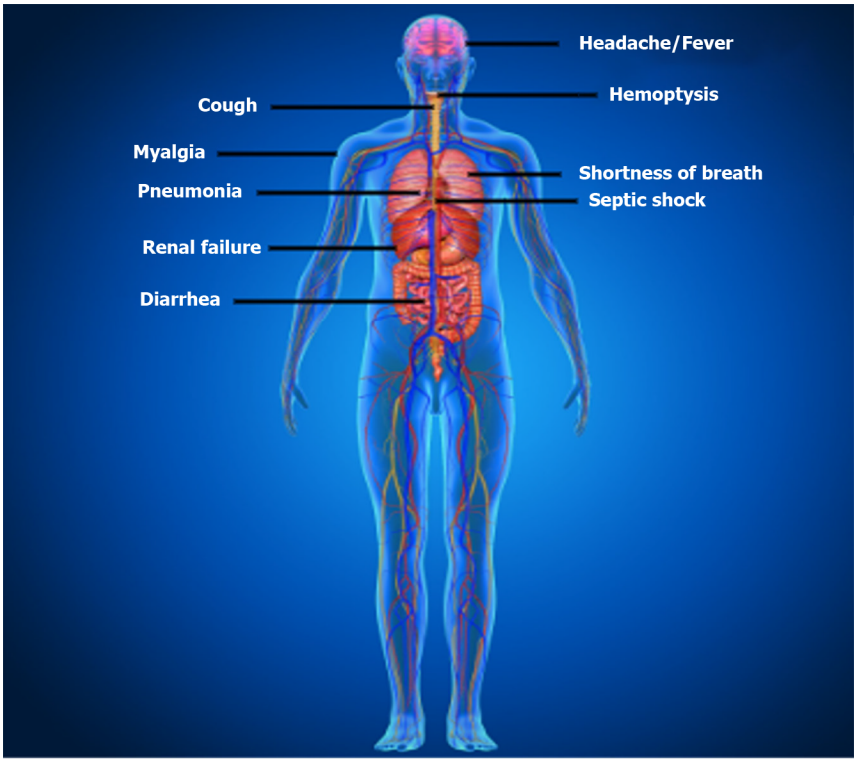
ACE2: Angiotensin converting enzyme 2.

Corticosteroids aid to escape ARDS and acute lung injury by lowering cytokine storm and lung inflammation[94]. Induced pluripotent stem cells, mesenchymal stromal cells, and T cells are various cell therapy techniques that have been researched[95-98].

PREVENTION

Currently, only a few approved medications are available to treat COVID-19 infection. Preventive measures play an important role to prevent this infection. It is advisable to keep confirmed or suspected cases having mild sickness isolated at home. Patients should wear a face mask and follow cough hygiene. Additionally, caregivers need to wash their hands regularly and should wear a surgical mask in the patient ward. Frequent sanitization of the rooms, surfaces, and equipment should be done with sodium hypochlorite. N95 respirators, safety suits, and goggles should be provided to healthcare professionals and workers. Healthcare professionals should also be frequently checked for various signs of COVID-19. Once a patient has been apyretic for at least three days and has two successive negative molecular tests with a sample gap of one day, they could be discharged from isolation. The only requirement for discharge was not the results of negative molecular tests[72].

Community-wide precautions include avoiding crowded places, forbidding large-scale gatherings, and delaying unnecessary travel to locations where transmission is still occurring. People should inculcate habit of good hand hygiene frequently, and exercise good cough hygiene by coughing into their sleeves or tissue paper rather than in their hands[99].



DOI: 10.13105/wjma.v11.i6.266

Figure 2 Clinical Features of patients with coronavirus disease 2019.

A law of banning the sale and trade of wild animals is also being introduced in China[100].

CONCLUSION

In this review, we outline the history, origin, transmission, genome structure, replication, epidemiology, pathogenesis, clinical characteristics, diagnosis, and treatment of COVID-19. The COVID-19 disease propagates rapidly across China and has disseminated to different countries of the world. Due to this viral epidemic, the economic, clinical, and public health frameworks of almost all countries of the world had affected. We wish that the horrible scenario created by this pandemic will not affect our life further.

ACKNOWLEDGEMENTS

The authors would like to thank Sh. Sanjay Kaushik, Lecturer (English), Government Model Sanskriti Senior Secondary School, Chulkana, Panipat, Haryana, India for timely support.

FOOTNOTES

Author contributions: Mokhria RK, Bhardwaj JK and Sanghi AK designed the outline, collected the data, wrote the manuscript, and proofread the paper.

Conflict-of-interest statement: All the author declare no conflict of interest for this article.

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S-Editor: Liu JH
L-Editor: A
P-Editor: Yu HG

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World Journal of Meta-Analysis

Submit a Manuscript: https://www.f6publishing.com

World J Meta-Anal 2023 September 18; 11(6): 277-289

DOI: 10.13105/wjma.v11.i6.277

ISSN 2308-3840 (online)

META-ANALYSIS

Endoscopic *vs* radiologic gastrostomy for enteral feeding: A systematic review and meta-analysis

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Homan M, Slovenia; Konishi H, Japan

Received: February 28, 2023
Peer-review started: February 28, 2023
First decision: March 24, 2023
Revised: May 17, 2023
Accepted: June 16, 2023
Article in press: June 16, 2023
Published online: September 18, 2023

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Abstract

BACKGROUND

Percutaneous endoscopic gastrostomy (PEG) and percutaneous radiological gastrostomy (PRG) are minimally invasive techniques commonly used for prolonged enteral nutrition. Despite safe, both techniques may lead to complications, such as bleeding, infection, pain, peritonitis, and tube-related complications. The literature is unclear on which technique is the safest.

AIM

To establish which approach has the lowest complication rate.

METHODS

A database search was performed from inception through November 2022, and comparative studies of PEG and PRG were selected following the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines. All included studies compared the two techniques directly and provided absolute values of the number of complications. Studies with pediatric populations were excluded. The primary outcome of this study was infection and bleeding. Pneumonia, peritonitis, pain, and mechanical complications were secondary outcomes. The risk of bias was assessed using the Cochrane risk-of-bias tool for randomized trials (RoB2) and we used The Risk of Bias in Nonrandomized Studies (ROBINS-I) to analyze the retrospective studies. We also performed GRADE analysis to assess the quality of evidence. Data on risk differences and

95% confidence intervals were obtained using the Mantel-Haenszel test.

RESULTS

Seventeen studies were included, including two randomized controlled trials and fifteen retrospective cohort studies. The total population was 465218 individuals, with 273493 having undergone PEG and 191725 PRG. The only outcome that showed a significant difference was tube related complications in retrospective studies favoring PEG (95%CI: 0.03 to 0.08; $P < 0.00001$), although this outcome did not show significant difference in randomized studies (95%CI: -0.07 to 0.04; $P = 0.13$). There was no difference in the analyses of the following outcomes: infection in retrospective (95%CI: -0.01 to 0.00; $P < 0.00001$) or randomized (95%CI: -0.06 to 0.04; $P = 0.44$) studies; bleeding in retrospective (95%CI: -0.00 to 0.00; $P < 0.00001$) or randomized (95%CI: -0.06 to 0.02; $P = 0.43$) studies; pneumonia in retrospective (95%CI: -0.04 to 0.00; $P = 0.28$) or randomized (95%CI: -0.09 to 0.11; $P = 0.39$) studies; pain in retrospective (95%CI: -0.05 to 0.02; $P < 0.00001$) studies; peritonitis in retrospective (95%CI: -0.02 to 0.01; $P < 0.0001$) studies.

CONCLUSION

PEG has lower levels of tube-related complications (such as dislocation, leak, obstruction, or breakdown) when compared to PRG.

Key Words: Gastrostomy; Adverse events; Meta-analysis; Percutaneous endoscopic; Radiological gastrostomy

Core Tip: Gastrostomy is a routine and preferred feeding route in patients who require enteral nutrition for prolonged period. This metanalysis compared percutaneous endoscopic gastrostomy and percutaneous radiological gastrostomy multiple outcomes, such as bleeding, infection, pneumonia, pain, and tube-related complications. Based on this meta-analysis, gastrostomy technique is related to a lower complication rate of tube-related complications and thus, should be preferred. Costs, devices availability, personal and local experience as well as patients preference should be considered when choose the best technique.

Citation: dos Santos ESV, de Oliveira GHP, de Moura DTH, Hirsch BS, Trasolini RP, Bernardo WM, de Moura EGH. Endoscopic *vs* radiologic gastrostomy for enteral feeding: A systematic review and meta-analysis. *World J Meta-Anal* 2023; 11(6): 277-289
URL: https://www.wjgnet.com/2308-3840/full/v11/i6/277.htm
DOI: https://dx.doi.org/10.13105/wjma.v11.i6.277

INTRODUCTION

Patients unable to tolerate oral intake for a prolonged period have an indication for an alternative route of enteral feeding, such as gastrostomy[1]. Gastrostomy involves connecting the stomach to an outflow in the skin with a tube, providing an alimentary route.

The first gastrostomy was performed in the 19th century, and Stamm's technique, surgical gastrostomy described in 1894, was long considered standard for performing a prolonged enteric access. The surgical technique became less performed with the emergence of the endoscopic technique. The method of percutaneous endoscopic gastrostomy (PEG) was first used in 1980 by Gauderer and Ponsky[2]. The technique was developed as a minimally invasive feeding route for neurologically impaired patients.

In 1981, percutaneous radiologic gastrostomy (PRG) was described[3], expanding the options available. This was an important development for scenarios such as head and neck tumors, where endoscopy is sometimes not an option, due to upper obstruction.

Endoscopic and radiological gastrostomy are both considered effective, safe and minimally invasive[4,5]. The preferred method is often based on specialist opinion or institution preference. We aim to perform a systematic review of the literature and meta-analysis to establish which approach has the lowest complication rate.

MATERIALS AND METHODS

Protocol and registration

This study was performed in conformity with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines[6] and was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the file number CRD42022377213.

Information source and literature search

The electronic databases searched were MEDLINE (via PubMed), Embase, Scopus, LILACS, the Cochrane Library (via BVS), and Google Scholar from inception until November 2022. The search was performed with the following mesh terms: [(Gastrostomy or Gastrostomies) and (Endoscopic)].

Eligibility criteria

The selection criteria were studies that contained patients undergoing gastrostomy, that compared the two interventions (PEG and PRG) and that included the following outcomes: Bleeding, infection, pain, peritonitis, tube-related complications with their results in absolute values.

Eligibility assessment was performed independently and standardized by 2 authors according to PRISMA guidelines [6]. Discrepancies between reviewers were resolved by consensus. A third reviewer was consulted in case of disagreements.

Case reports, reviews and letters were excluded. Studies that exclusively analyzed patients under 18 years of age, compared other techniques or did not consider the desired outcomes were excluded. Studies with the pediatric population were excluded because of anatomical differences with the adult population and consequently different complications.

To assess the quality of eligible studies we used The Risk of Bias in Nonrandomized Studies (ROBINS-I)[7] to analyze the comparative studies and the Cochrane risk-of-bias tool for randomized trials (RoB2)[8] to analyze the randomized studies. The quality of evidence was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) criteria using the GRADE pro Guideline Development Tool software (Mc Master University, Ontario, Canada)[9].

Data analyses

The randomized controlled trials (RCT) studies were analyzed separately from the observational studies since they have different levels of evidence. This allowed us to compare the outcomes separately and to make a global analysis of the results.

The analysis was performed using Review Manager (RevMan 5.4) from the Cochrane Informatics & Knowledge Management Department website. Risk differences for dichotomous variables were computed using a fixed-effects model and the respective forest and funnel plots were obtained. Data on risk differences and the 95% confidence intervals (CI) for each outcome were calculated using the Mantel-Haenszel test. Inconsistency (heterogeneity) was qualified and reported using the Chi-squared (Chi^2) and Higgins methods and was termed I^2. I^2 values > 50% were considered to indicate substantial heterogeneity. We performed an analysis using a funnel plot to identify possible outliers. If the sample became homogeneous after excluding possible outliers, the studies were permanently excluded. We used random effects to reduce the influence of heterogeneity on the final result[10]. Outcome measures are described as the mean difference or risk difference (RD), with their corresponding 95%CI.

RESULTS

The initial search showed 15585 results, after removing the duplicate articles, 6490 remained. A total of twenty studies passed the screening stage and were included in qualitative synthesis, seventeen studies met criteria to be included in the metanalysis, two were prospective randomized studies and fifteen were retrospective cohort studies. The search strategy can be visualized in the following diagram (Figure 1).

Study characteristics

Seventeen studies were included in the systematic review, including two RCTs, one prospective, and 14 retrospective cohort studies. A total of 465218 individuals, with 273493 received PEG and 191725 PRG. The characteristics of the studies can be seen in Table 1[11-27]. Early outcomes were analyzed.

Risk of bias within studies

The ROBINS-I and ROB-2 scoring system were used to evaluate risk of bias for observational[12-18,20-27] and randomized studies[11,19], respectively (Table 1). We identified a low risk of bias in the two RCT studies (Figure 2), and a strong methodological quality. As for the observational studies, we note that 5 of them present serious risk of bias[13,15,25,27] and 5 moderate risk[12,14,18,21,23], mostly due to issues in the dissemination of results (Figure 3).

Quality of evidence

The objective criteria of GRADE analysis to evaluate the quality of evidence identified moderate certainty for pain and infection, low certainty for peritonitis and very low certainty for bleeding and pneumonia (Figure 4).

Infection

A total of 465198 patients from 17 studies[12-27] were analyzed. There was no difference in the incidence of infection in retrospective (95%CI: -0.01 to 0.00; $P < 0.00001$; $I^2 = 74\%$) or randomized (95%CI: -0.06 to 0.04; $P = 0.68$; $I^2 = 0\%$) studies. In the overall analysis there was no difference in the meta-analysis of observational and RCT studies combined (95%CI: -0.01 to 0.00; $P = 0.56$; $I^2 = 70\%$) (Figure 5A).

Table 1 Early outcomes were analyzed

Ref.	Country	Design	Period	PEG (N)	RIG (N)	Mean age PEG	Mean age RIG	Single (S) or Multicenter (M)
Hoffer *et al*[11], 1999	United States	Randomized	1993-1994	69	66	58.2	51.9	S
Möller *et al*[12], 1999	Sweden	Retrospective	1990-1994	12	94	48	64	S
Laasch *et al*[13], 2002	United Kingdom	Prospective	2000-2002	50	50	73	68	M (3)
Silas *et al*[14], 2005	United States	Retrospective	1997-2001	177	193	68	63	S
Rustom *et al*[15], 2006	United Kingdom	Retrospective	2002-2005	40	28	63.6	64.8	S
Galaski *et al*[16], 2009	Canada	Retrospective	2004-2005	30	44	55	65	S
La Nauze *et al*[17], 2012	Australia	Retrospective	2007-2009	80	97	61	61	S
Rio *et al*[18], 2010	United Kingdom	Retrospective	1999-2006	21	122	64	64	S
Lewis *et al*[19], 2014	United Kingdom	Randomized	2012-2013	34	31	73	71	S
ProGas Study Group[20], 2015	United Kingdom	Retrospective	2010-2014	121	163	64.2	63.6	M (24)
Vidhya *et al*[21], 2018	Australia	Retrospective	2013-2015	85	52	65	64	S
Park *et al*[22], 2019	South Korea	Retrospective	2010-2015	324	94	66	66.2	M (5)
Strijbos *et al*[23], 2019	Netherlands	Retrospective	2008-2016	291	469	66	66.2	S
Lainez *et al*[24], 2020	Spain	Retrospective	2019	25	23	63.98	62.41	S
Maasarani *et al*[25], 2020	United States	Retrospective	2004-2014	232164	26477	NI	NI	M
Kohli *et al*[26], 2020	United States	Retrospective	2014-2017	16384	154007	53.7	67.2	M
Kohli *et al*[27], 2021	United States	Retrospective	2011-2021	23566	9715	70.7	69.6	M

PEG: Percutaneous endoscopic gastrostomy; PRG: Radiologically guided gastrostomy; NI: Not informed.

Bleeding

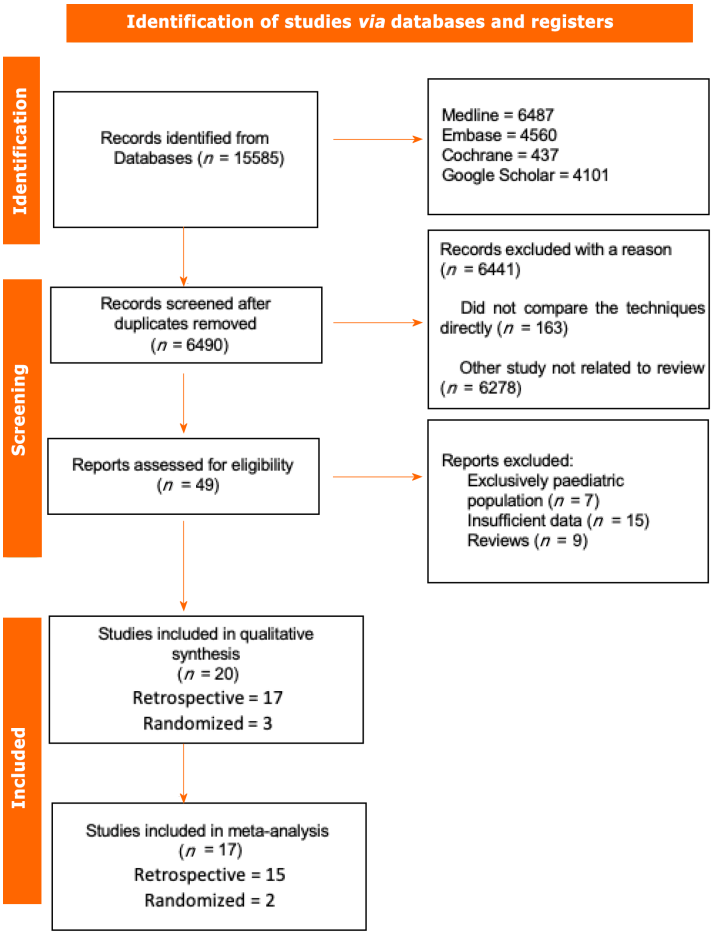
A total of 464618 patients from fourteen[11-13,16,17,19-27] studies were analyzed. There was no difference in the incidence of bleeding in observational studies (95% CI: -0.00 to 0.00; $P < 0.00001$; $I^2 = 76\%$) or RCTs (95%CI: -0.06 to 0.02; $P = 0.43$; $I^2 = 0\%$). In the overall analysis there was no difference in the meta-analysis of observational and RCT studies combined (95%CI: -0.00 to 0.00); $P = 0.81$; $I^2 = 73\%$) (Figure 5B).

Pneumonia

A total of 1796 patients from eight[11,13,17,19-21,23,24] studies were analyzed. There was no difference in the incidence of pneumonia in comparative studies (95%CI: -0.00 to 0.04; $P = 0.28$; $I^2 = 20\%$) or RCT (95%CI: -0.10 to 0.10; $P = 0.39$; $I^2 = 0\%$) studies. In the overall analysis there was no difference in the meta-analysis of observational and RCT studies combined (95%CI: -0.00 to 0.03; $P = 0.44$; $I^2 = 0\%$) (Figure 5C).

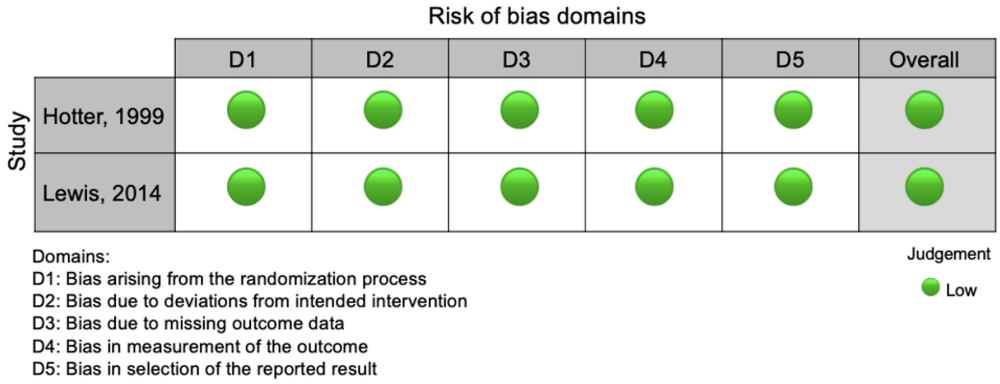
Peritonitis

A total of 34461 patients from five[12,17,21,23,27] were analyzed. There was no difference in the incidence of peritonitis in retrospective (95%CI: -0.02 to 0.01; $P < 0.0001$; $I^2 = 86\%$) studies. It was not possible to evaluate the peritonitis outcome in RCT studies because this outcome was not included in these studies (Figure 5D).



DOI: 10.13105/wjma.v11.i6.277

Figure 1 Flow diagram showing study selection process.



DOI: 10.13105/wjma.v11.i6.277

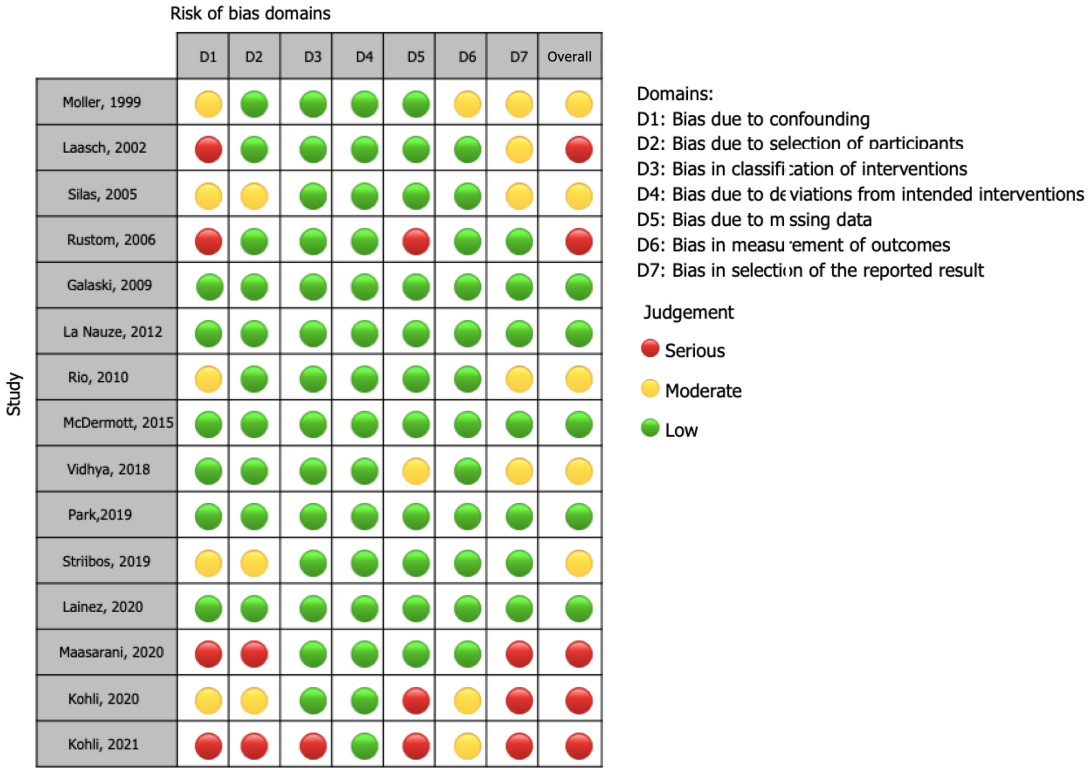
Figure 2 Risk of bias according to ROB-2.

Pain

A total of 260793 patients from seven[14,17,18,20,22,23,25] studies were analyzed. There was no difference in the incidence of pain in retrospective (95%CI: -0.05 to 0.02; $P < 0.00001$; $I^2 = 91\%$) studies. It was not possible to evaluate the pain outcome in RCT studies because this outcome was not included in these studies (Figure 5E).

Tube related complications

A total of 464689 patients from 14 studies[11-19,21-23,25,26] were analyzed. This analysis showed a significant difference in tube related complications in observational studies favoring PEG (95%CI: -0.03 to -0.08; $P < 0.00001$), although there was no significant difference in randomized studies (95%CI: -0.07 to 0.04; $P = 0.13$). In the global analysis there was a difference, favoring PEG (95%CI: -0.07 to -0.03; $P < 0.00001$) (Figure 6).



DOI: 10.13105/wjma.v11.i6.277

Figure 3 Risk of bias according to ROBINS-I.

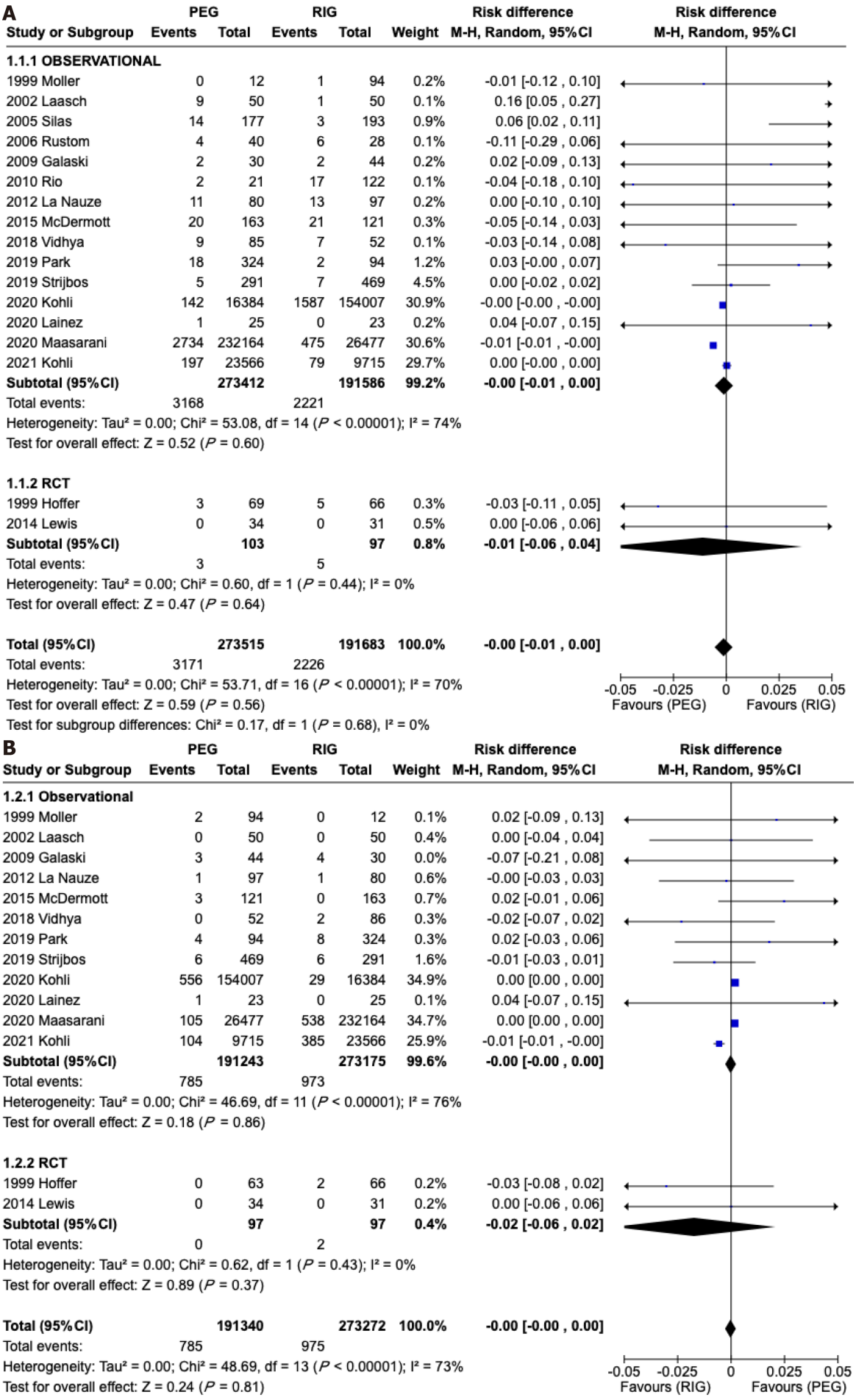
Certainty assessment							N° of patients		Effect		Certainty
N° of studies	Study desing	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Pain	Placebo	Relative (95% CI)	Absolute (95% CI)	
Pain											
7	observational studies	not serious	not serious	serious	not serious	none	12230/27573 (44.4%)	96205/233220 (41.3%)	**RR 1.11** (1.09 to 1.12)	**45 more per** 1.000 (from 37 more to 50 more)	◉◉◉◎ Moderate
Infection											
17	observational studies	not serious	serious	not serious	not serious	none	2226/191683 (1.2%)	3171/273515 (1.2%)	**RR 1.10** (0.87 to 1.38)	**1 more per** 1.000 (from 2 fewer to 4 more)	◉◉◉◎ Moderate
Peritonitis											
5	observational studies	not serious	not serious	not serious	not serious	none	28/10427 (0.3%)	455/24034 (1.9%)	**RR 0.54** (0.11 to 2.56)	**9 fewer per** 1.000 (from 17 fewer to 30 more)	◉◉◎◎ Low
Bleeding											
14	observational studies	not serious	serious	not serious	not serious	none	785/191277 (0.4%)	973/273206 (0.4%)	**RR 1.16** (0.69 to 1.95)	**1 more per** 1.000 (from 1 fewer to 3 more)	◉◎◎◎ Very low
Pneumonia											
8	observational studies	serious	not serious	not serious	not serious	none	28/909 (3.1%)	39/797 (4.9%)	**RR 0.72** (0.46 to 1.14)	**14 fewer per** 1.000 (from 26 fewer to 7 more)	◉◎◎◎ Very low

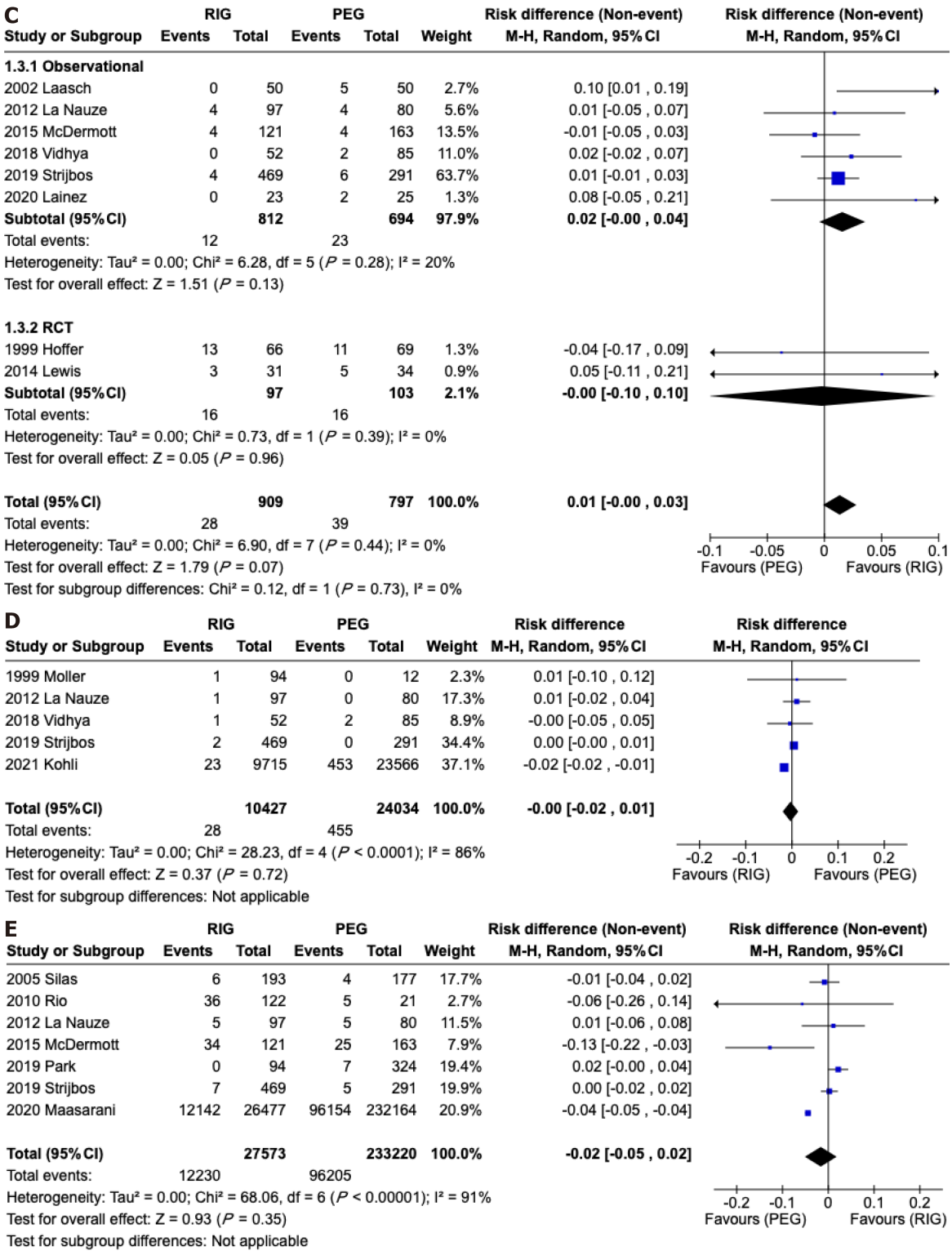
DOI: 10.13105/wjma.v11.i6.277

Figure 4 Quality of evidence assessed by Grading of Recommendations Assessment, Development, and Evaluation.

DISCUSSION

This meta-analysis shows that both PEG and PRG techniques are similar in terms of safety profile, except potentially in tube-related complications, which was higher for PRG in observational studies (Evidence 2A). We included 20 studies in this review (3 randomized and 17 comparative studies) and 17 in our meta-analysis, totaling 465218 individuals, with 273493 undergoing PEG and 191725 undergoing PRG. While other metanalyses compared these 2 approaches[28-34], this analysis is unique as it includes the largest number of adult patients and also separates RCT and observational studies providing further insight. This approach follows Cochrane recommendations and thus provides for a more reliable


A
PEG
RIG
Risk difference
Risk difference
Study or Subgroup Events Total Events Total Weight M-H, Random, 95%CI
M-H, Random, 95%CI
1.1.1 OBSERVATIONAL
1999 Moller 0 12 1 94 0.2% -0.01 [-0.12 , 0.10]
2002 Laasch 9 50 1 50 0.1% 0.16 [0.05 , 0.27]
2005 Silas 14 177 3 193 0.9% 0.06 [0.02 , 0.11]
2006 Rustom 4 40 6 28 0.1% -0.11 [-0.29 , 0.06]
2009 Galaski 2 30 2 44 0.2% 0.02 [-0.09 , 0.13]
2010 Rio 2 21 17 122 0.1% -0.04 [-0.18 , 0.10]
2012 La Nauze 11 80 13 97 0.2% 0.00 [-0.10 , 0.10]
2015 McDermott 20 163 21 121 0.3% -0.05 [-0.14 , 0.03]
2018 Vidhya 9 85 7 52 0.1% -0.03 [-0.14 , 0.08]
2019 Park 18 324 2 94 1.2% 0.03 [-0.00 , 0.07]
2019 Strijbos 5 291 7 469 4.5% 0.00 [-0.02 , 0.02]
2020 Kohli 142 16384 1587 154007 30.9% -0.00 [-0.00 , -0.00]
2020 Lainez 1 25 0 23 0.2% 0.04 [-0.07 , 0.15]
2020 Maasarani 2734 232164 475 26477 30.6% -0.01 [-0.01 , -0.00]
2021 Kohli 197 23566 79 9715 29.7% 0.00 [-0.00 , 0.00]
Subtotal (95%CI) 273412 191586 99.2% -0.00 [-0.01 , 0.00]
Total events: 3168 2221
Heterogeneity: Tau² = 0.00; Chi² = 53.08, df = 14 (P < 0.00001); I² = 74%
Test for overall effect: Z = 0.52 (P = 0.60)
1.1.2 RCT
1999 Hoffer 3 69 5 66 0.3% -0.03 [-0.11 , 0.05]
2014 Lewis 0 34 0 31 0.5% 0.00 [-0.06 , 0.06]
Subtotal (95%CI) 103 97 0.8% -0.01 [-0.06 , 0.04]
Total events: 3 5
Heterogeneity: Tau² = 0.00; Chi² = 0.60, df = 1 (P = 0.44); I² = 0%
Test for overall effect: Z = 0.47 (P = 0.64)
Total (95%CI) 273515 191683 100.0% -0.00 [-0.01 , 0.00]
Total events: 3171 2226
Heterogeneity: Tau² = 0.00; Chi² = 53.71, df = 16 (P < 0.00001); I² = 70%
Test for overall effect: Z = 0.59 (P = 0.56)
Test for subgroup differences: Chi² = 0.17, df = 1 (P = 0.68), I² = 0%
-0.05 -0.025 0 0.025 0.05
Favours (PEG) Favours (RIG)
B
PEG
RIG
Risk difference
Risk difference
Study or Subgroup Events Total Events Total Weight M-H, Random, 95%CI
M-H, Random, 95%CI
1.2.1 Observational
1999 Moller 2 94 0 12 0.1% 0.02 [-0.09 , 0.13]
2002 Laasch 0 50 0 50 0.4% 0.00 [-0.04 , 0.04]
2009 Galaski 3 44 4 30 0.0% -0.07 [-0.21 , 0.08]
2012 La Nauze 1 97 1 80 0.6% -0.00 [-0.03 , 0.03]
2015 McDermott 3 121 0 163 0.7% 0.02 [-0.01 , 0.06]
2018 Vidhya 0 52 2 86 0.3% -0.02 [-0.07 , 0.02]
2019 Park 4 94 8 324 0.3% 0.02 [-0.03 , 0.06]
2019 Strijbos 6 469 6 291 1.6% -0.01 [-0.03 , 0.01]
2020 Kohli 556 154007 29 16384 34.9% 0.00 [0.00 , 0.00]
2020 Lainez 1 23 0 25 0.1% 0.04 [-0.07 , 0.15]
2020 Maasarani 105 26477 538 232164 34.7% 0.00 [0.00 , 0.00]
2021 Kohli 104 9715 385 23566 25.9% -0.01 [-0.01 , -0.00]
Subtotal (95%CI) 191243 273175 99.6% -0.00 [-0.00 , 0.00]
Total events: 785 973
Heterogeneity: Tau² = 0.00; Chi² = 46.69, df = 11 (P < 0.00001); I² = 76%
Test for overall effect: Z = 0.18 (P = 0.86)
1.2.2 RCT
1999 Hoffer 0 63 2 66 0.2% -0.03 [-0.08 , 0.02]
2014 Lewis 0 34 0 31 0.2% 0.00 [-0.06 , 0.06]
Subtotal (95%CI) 97 97 0.4% -0.02 [-0.06 , 0.02]
Total events: 0 2
Heterogeneity: Tau² = 0.00; Chi² = 0.62, df = 1 (P = 0.43); I² = 0%
Test for overall effect: Z = 0.89 (P = 0.37)
Total (95%CI) 191340 273272 100.0% -0.00 [-0.00 , 0.00]
Total events: 785 975
Heterogeneity: Tau² = 0.00; Chi² = 48.69, df = 13 (P < 0.00001); I² = 73%
Test for overall effect: Z = 0.24 (P = 0.81)
Test for subgroup differences: Chi² = 0.78, df = 1 (P = 0.38), I² = 0%
-0.05 -0.025 0 0.025 0.05
Favours (RIG) Favours (PEG)



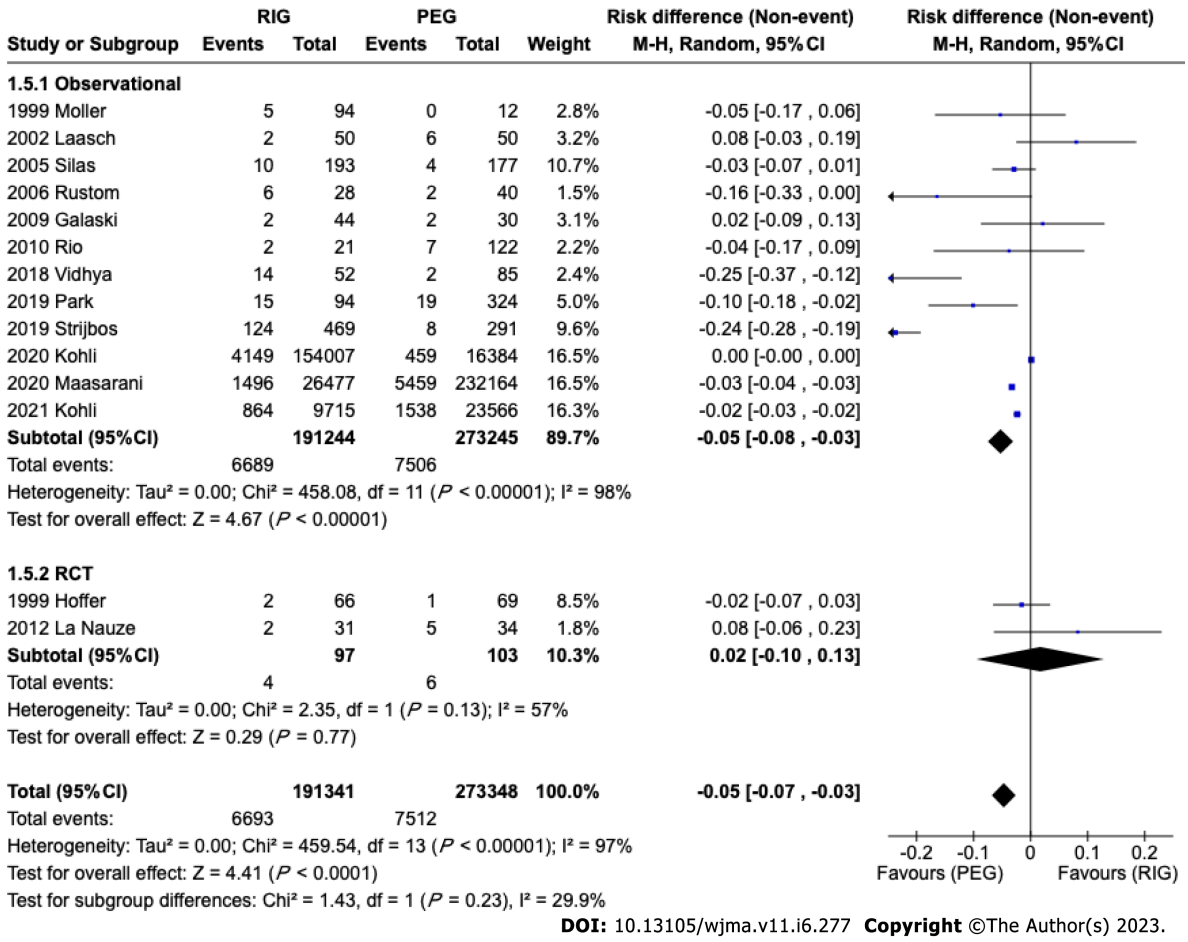
DOI: 10.13105/wjma.v11.i6.277

Figure 5 Forest plot studies reporting. A: Outcomes infection; B: Outcomes bleeding; C: Pneumonia; D: Outcomes peritonitis; E: Pain.

comparison. Additionally, we separated all adverse events, including pain and pneumonia, which have not been individually analyzed to date. The adverse effects chosen were based on previous publications showing the most frequent complications related to the method[4].

The three most common techniques for performing gastrostomy are endoscopic, radiologic, and surgical. Although surgical gastrostomy was the first described approach, it is now less used due to its invasiveness. A meta-analysis including RCT (evidence 1A) comparing endoscopic and surgical techniques demonstrated a lower number of minor complications for endoscopic procedures[35].

Until now, there is no consensus regarding the superiority of either endoscopic or radiologic gastrostomy. Our results clarify that both approaches are similar in terms of safety as shown in our meta-analysis including only RCTs.



DOI: 10.13105/wjma.v11.i6.277

Figure 6 Forest plot with studies reporting tube related complications.

Furthermore, a recent RCT including 42 patients comparing the two techniques[36], showed similar results to this meta-analysis. Unfortunately, this RCT was not included due to a lack of data available in the published manuscript, despite our attempt to contact the author.

Local infection is a common adverse outcome of gastrostomy. For this reason, the American Society for Gastrointestinal Endoscopy[37] and the Society for Interventional Radiology[38,39] recommends administering periprocedural antibiotics. The studies utilized in this meta-analysis did not expressly state if antibiotics were administered or not, but as this is a common practice, it was likely used. Our meta-analysis did not demonstrate a significant difference regarding infection in both RCT and non-RCT analysis.

In previous publications[26,27], it has been stated that patients undergoing PEG have a higher rate of bleeding since PEG is preferentially performed in patients with diseases requiring antiplatelets or anticoagulants such as stroke and vascular dementia[27,40]. We expected to prove this hypothesis, however, this meta-analysis demonstrated a low risk of bleeding due to the gastrostomy procedure, without a statistically significant difference between PEG and PRG in both RCT and observational studies. Data on antiplatelet and/or anticoagulant medications among patients who bled were not available.

This study showed no significant difference in the incidence of pneumonia. In previous studies it was observed that gastrostomy compared to nasogastric feeding has a lower incidence of pneumonia, however, this complication is a major cause of mortality in patients undergoing gastrostomy[16]. It is important to state that we were not able to evaluate gastrostomy and gastrojejunostomy separately due to a lack of data. Gastrojejunostomy is associated with a theoretically lower rate of reflux and pneumonia[11,19].

Pain and peritonitis are complex outcomes to measure objectively. Since the definition of these outcomes differs in several studies[13,14,17,18,20-25]. There was no statistical difference between the two methods in our study.

In the analyzed studies, the types, brands, and sizes of tubes were not differentiated. This heterogeneity may influence the results of this analysis. The meta-analysis of observational studies demonstrated a statistically significant difference in the incidence of tube-related complications of a PEG and PRG, such as dislocation, leak, obstruction, or breakdown, showing a higher incidence in PRG. In the RCT meta-analysis, there was no difference. However, the observational studies included 464489 patients versus 200 patients from RCT studies and this should be considered if the RCTs were underpowered to detect a small difference between the techniques. A difference may be expected due to the size difference between endoscopic and radiological techniques. PEG is usually performed using 20FR or 24FR tubes whereas PRG uses 14-16 FR[41]. The size of the gastrostomy ostium influences the incidence of migration; a smaller caliber is associated with a higher incidence of migration and obstruction. The feeding tube can become blocked due to various

reasons, such as the accumulation of food formula, medications, or debris. Smaller tubes increase the probability of the tube becoming blocked. Leaks can occur around the insertion site or through the tube itself, which can cause skin irritation and infection, so if the size of the skin insertion is larger than the tube caliber there is a greater chance of leakage.

Tube-related complications are usually associated with longer hospital stays, the need for further procedures, and potentially increased costs[16,33,42]. Evaluating costs is challenging since procedure cost varies significantly between countries. A study comparing the two techniques published in 2009 showed that the costs of the procedures are also different, with PEGs being 43% more expensive than PRGs[16] but the costs are related only to the procedure and not to the overall cost. In Brazil, PEG has a low cost, being more cost-effective than a CT scan. Although few studies provide information regarding costs, this information would be useful, given that these procedures are performed on a large scale worldwide[11,16].

The strengths of this study include a large number of patients from different continents, dedicated analysis of RCT data, use of a validated quality assessment tool, and application of the GRADE process to assess the quality of our data.

Although systematic review and meta-analysis represent the most thorough assessment of available evidence comparing the risks of PEG and PRG, our study has limitations as discussed above. Most data was gathered from observational studies. Additionally, lack of data on tube size, antibiotic, and anticoagulant use, indications for the gastrostomy procedure, and inclusion of both gastrostomy and gastrojejunostomy all limit understanding of potential nuances that differentiate PEG from PRG.

In summary, both approaches are safe. Thus, individual evaluation is required considering several factors including local and personal experience, device availability, cost, and patient preference.

CONCLUSION

PEG and PRG present a similar safety profile. However, PRG is associated with a slightly higher rate of tube-related complications, potentially related to the small caliber of the gastrostomy tube.

ARTICLE HIGHLIGHTS

Research background

Gastrostomy feeding is superior to nasogastric tube feeding when medium to long-term enteral feeding (≥ 4 wk) is indicated. The optimal technique for long-term enteral feeding is not yet well established. Therefore, we performed a meta-analysis comparing the two methods.

Research motivation

This paper motivation is to demonstrate which technique for performing a gastrostomy has the lowest incidence rate of adverse events.

Research objectives

The aim of the paper is to compare the technique of endoscopic gastrostomy (PEG) and gastrostomy *via* interventional radiology (PRG) and establish which technique is the safest for the patient.

Research methods

Comparative studies of PEG and PRG were selected. Included studies had outcomes such as infection, bleeding, pneumonia, pain, peritonitis and tube related complications. The risk of bias and quality of evidence were assessed. The analysis was performed using Review Manager (RevMan 5.4) from the Cochrane Informatics & Knowledge Management Department website.

Research results

Seventeen studies were included, with a total of 465218 patients. The only outcome that showed a significant difference was tube-related complications in retrospective studies favoring PEG (95%CI: 0.03 to 0.08; $P < 0.00001$), although this outcome did not show significant difference in randomized studies (95%CI: -0.07 to 0.04; $P = 0.13$). There was no difference in the analyses of the following outcomes: Infection in retrospective (95%CI: -0.01 to 0.00; $P < 0.00001$) or randomized (95%CI: -0.06 to 0.04; $P = 0.44$) studies; bleeding in retrospective (95%CI: -0.00 to 0.00; $P < 0.00001$) or randomized (95%CI: -0.06 to 0.02; $P = 0.43$) studies; pneumonia in retrospective (95%CI: -0.04 to 0.00; $P = 0.28$) or randomized (95%CI: -0.09 to 0.11; $P = 0.39$) studies; pain in retrospective (95%CI: -0.05 to 0.02; $P < 0.00001$) studies; peritonitis in retrospective (95%CI: -0.02 to 0.01; $P < 0.0001$) studies.

Research conclusions

The study concluded that RIG has a higher incidence of tube-related complications than PEG. This difference is probably associated with the caliber of the tubes used. There was no statistical difference in the other outcomes evaluated.

Research perspectives

This study aimed to determine which technique is safer for the patient, and both methods proved to be safe. We can conclude that the choice of technique depends on the type of patient, the experience of the service, the cost, and the availability of the method.

FOOTNOTES

Author contributions: dos Santos ESV contributed acquisition of data, analysis, interpretation of data, drafting the article, revising the article, final approval; de Oliveira GHP, dos Santos ESV and Hirsch BS contributed analysis and interpretation of data, revising the article; de Moura DTH contributed analysis of data, interpretation of data, drafting the article, revising the article, final approval; Bernardo WM contributed analysis of data, interpretation of data, drafting the article, revising the article, final approval; de Moura EGH contributed analysis and interpretation of data, drafting the article, revising the article, final approval.

Conflict-of-interest statement: The authors deny any conflict of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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S-Editor: Liu JH
L-Editor: A
P-Editor: Zhao S

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World Journal of Meta-Analysis

Submit a Manuscript: https://www.f6publishing.com

World J Meta-Anal 2023 September 18; 11(6): 290-312

DOI: 10.13105/wjma.v11.i6.290

ISSN 2308-3840 (online)

META-ANALYSIS

Evidence relating cigarettes, cigars and pipes to cardiovascular disease and stroke: Meta-analysis of recent data from three regions

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Specialty type: Public, environmental and occupational health

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A, A
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Arboix A, Spain; Han J, China; Skrlec I, Croatia

Received: June 23, 2023
Peer-review started: June 23, 2023
First decision: August 4, 2023
Revised: August 14, 2023
Accepted: August 21, 2023
Article in press: August 21, 2023
Published online: September 18, 2023

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Abstract

BACKGROUND

More recent data are required relating to disease risk for use of various smoked products and of other products containing nicotine. Earlier we published meta-analyses of recent results for chronic obstructive pulmonary disease and lung cancer on the relative risk (RR) of current compared to never product use for cigarettes, cigars and pipes based on evidence from North America, Europe and Japan. We now report corresponding up-to-date evidence for acute myocardial infarction (AMI), ischaemic heart disease (IHD) and stroke.

AIM

To estimate, using recent data, AMI, IHD and stroke RRs by region for current smoking of cigarettes, cigars and pipes.

METHODS

Publications in English from 2015 to 2020 were considered that, based on epidemiological studies in the three regions, estimated the current smoking RR of AMI, IHD or stroke for one or more of the three products. The studies should involve at least 100 cases of stroke or cardiovascular disease (CVD), not be restricted to populations with specific medical conditions, and should be of cohort or nested case-control study design or randomized controlled trials. A literature search was conducted on MEDLINE, examining titles and abstracts initially, and then full texts. Additional papers were sought from reference lists of selected papers, reviews and meta-analyses. For each study identified, we entered the most recent available data on current smoking of each product, as well as the characteristics of the study and the RR estimates. Combined RR estimates were derived using random-effects meta-analysis for stroke and, in the case of CVD, separately for IHD and AMI. For cigarette smoking, where far more data were available, heterogeneity was studied by a wide range of factors. For cigar and pipe smoking, a more limited heterogeneity analysis was carried out. A more limited assessment of variation in risk by daily number of cigarettes smoked was also conducted.

Results were compared with those from previous meta-analyses published since 2000.

RESULTS

Current cigarette smoking: Ten studies gave a random-effects RR for AMI of 2.72 [95% confidence interval (CI): 2.40-3.08], derived from 13 estimates between 1.47 and 4.72. Twenty-three studies gave an IHD RR of 2.01 (95%CI: 1.84-2.21), using 28 estimates between 0.81 and 4.30. Thirty-one studies gave a stroke RR of 1.62 (95%CI: 1.48-1.77), using 37 estimates from 0.66 to 2.91. Though heterogeneous, only two of the overall 78 RRs were below 1.0, 71 significantly ($P < 0.05$) exceeding 1.0. The heterogeneity was only partly explicable by the factors studied. Estimates were generally higher for females and for later-starting studies. They were significantly higher for North America than Europe for AMI, but not the other diseases. For stroke, the only endpoint with multiple Japanese studies, RRs were lower there than for Western studies. Adjustment for multiple factors tended to increase RRs. Our RR estimates and the variations by sex and region are consistent with earlier meta-analyses. RRs generally increased with amount smoked. Current cigar and pipe smoking: No AMI data were available. One North American study reported reduced IHD risk for non-exclusive cigar or pipe smoking, but considered few cases. Two North American studies found no increased stroke risk with exclusive cigar smoking, one reporting reduced risk for exclusive pipe smoking (RR 0.24, 95%CI: 0.06-0.91). The cigar results agree with an earlier review showing no clear risk increase for IHD or stroke.

CONCLUSION

Current cigarette smoking increases risk of AMI, IHD and stroke, RRs being 2.72, 2.01 and 1.62. The stroke risk is lower in Japan, no increase was seen for cigars/pipes.

Key Words: Cigarettes; Cigars; Pipes; Cardiovascular disease; Stroke; Meta-analysis; Review

Core Tip: Recent North American and European studies indicate that current, compared to never cigarette smoking, increases risk in each sex by about 3-fold for acute myocardial infarction, about 2-fold for ischaemic heart disease (IHD), and about 1.6-fold for stroke. More limited evidence from Japanese studies suggests a similar increase in risk for IHD, but a lower increase, of about 1.2-fold, for stroke. The increase in risk is greater in heavier smokers. Limited recent data for cigar or pipe smoking, all from North America, finds no evidence of an increased risk of IHD or stroke, one study reporting a significantly reduced risk of stroke in exclusive pipe smokers. Our findings are generally consistent with evidence from earlier studies. Cigarette smoking increases risk of all the three diseases studied, but by a much smaller factor than noted for lung cancer and chronic obstructive pulmonary disease in our companion publication. Any increase in risk from cigar and pipe smoking has not been demonstrated.

Citation: Lee PN, Coombs KJ, Hamling JS. Evidence relating cigarettes, cigars and pipes to cardiovascular disease and stroke: Meta-analysis of recent data from three regions. *World J Meta-Anal* 2023; 11(6): 290-312
URL: https://www.wjgnet.com/2308-3840/full/v11/i6/290.htm
DOI: https://dx.doi.org/10.13105/wjma.v11.i6.290

INTRODUCTION

It is known that cigarette smoking increases risk of various diseases, particularly chronic obstructive pulmonary disease (COPD), lung cancer, stroke and various forms of cardiovascular disease (CVD), including ischaemic heart disease (IHD) and acute myocardial infarction (AMI)[1,2]. However, any risk increases from cigar or pipe smoking, or from using other products containing nicotine are less well investigated. In a project based on studies conducted in North America, Europe and Japan (regions commonly studied in predictive modelling exercises[3-8] and which do not include countries such as India, where a wide variety of other tobacco products are commonly used), we are comparing relative risks (RRs) of various diseases for current *vs* never use of different products. In this journal we earlier published two reviews with meta-analyses of recent epidemiological evidence. One related current use of snus (Swedish snuff) or smokeless tobacco to risk of the major smoking-related diseases[9]. Another related current cigarette, pipe and cigar smoking to risk of lung cancer and COPD[10]. Here we systematically review and meta-analyse evidence relating current smoking of cigarettes, pipes and cigars to risk of AMI, IHD and stroke, based on publications in 2015 to 2020. We do not consider either electronic cigarettes or heat-not-burn products in our project, because large long-term studies relating risk of the main smoking-related diseases to their use have not so far been conducted. As in our previous publications we aim only to carry out meta-analyses concerning current product use, and to study how the derived RRs vary by factors like sex and region, and not investigating in detail variation by amount smoked, duration of smoking, time quit, or age at onset.

The work described here partially updates two earlier meta-analyses of ours. One[5], based on data from 15 countries in Europe, Asia or North America, reported analyses comparing risk in current v never cigarette smoking, giving a RR of 2.05 (95%CI: 1.90-2.21) combining 92 estimates for IHD/AMI, and of 1.48 (95%CI: 1.37-1.60) combining 57 estimates for stroke. The other[11], limited to Japan, gave an RR of 2.21 (95%CI: 1.96-2.50) combining 20 estimates for IHD and of 1.40 (95%CI: 1.25-1.57) combining 16 estimates for stroke. Neither of these reviews considered cigar or pipe smoking specifically. We compare our derived RR estimates with those earlier results, and also with findings of other meta-analyses/reviews published between 2000 and 2020, some of IHD and stroke[12-18], one of IHD only[19], some of stroke only[20-23] and some limited to particular types of stroke[24-28]. These reviews generally relate to cigarette smoking, or to undefined smoking, but one[12] gives results for exclusive cigar smokers.

MATERIALS AND METHODS

Study inclusion and exclusion criteria

Attention was restricted to publications in English in the years 2015 to 2020 which provided RR estimates for stroke, IHD or AMI comparing current and never smokers of cigarettes, of cigars, or of pipes. These had to be based on epidemiological cohort or nested case-control studies or randomized controlled trials which were conducted in North America, Europe or Japan, and which involved at least 100 cases of the disease of interest. The studies were excluded if they were restricted to specific types of the diseases, or to patients with specific medical conditions, or if the results were superseded by corresponding later results from the same study. Studies providing estimates for equivalent diseases, such as cerebrovascular disease rather than stroke, coronary heart (or artery) disease rather than IHD, or myocardial infarction rather than AMI were also included. However, studies providing estimates only for disease subsets, such as specific types of stroke were not included.

Literature searches

Initially, at stage 0, literature searches were conducted on MEDLINE for publications in 2015 to 2020. Searches were carried out on November 13, 2021 and used the terms "smoking" OR "smoking [MeSH Major Topic]" AND "cardiovascular disease" OR "heart disease" OR "stroke".

Then, at stage 1, titles and abstracts were screened to select publications that appeared to describe studies satisfying the inclusion criteria, and both meta-analyses and reviews that may cite other relevant publications. The initial screening was usually carried out by PNL, with acceptances checked by KJC, though in some cases KJC did the initial screening and PNL the checking. Disagreements were resolved *via* discussion.

Then, at stage 2, the full texts of the selected publications (and of relevant Supplementary files and other publications linked to them in the MEDLINE search) were obtained, and examined by PNL, who classified the publication as being an acceptance (*i.e.* it appeared to include relevant data), a reject (giving reason), a relevant review or a relevant meta-analysis. The rejections were then checked by KJC, with any disagreements resolved.

At stage 3, additional accepted publications not detected by the MEDLINE searches were sought by examination of reference lists of the accepted papers and of the relevant reviews and meta-analyses and, when obtained, dealt with as in stage 2.

Finally, at stage 4, copies of all the accepted publications (not the meta-analyses) were organized, first by country, and then by study within country, with studies conducted in multiple countries considered as a separate group. The aim was to eliminate from consideration those publications giving results for a study that were superseded by a later publication, and those publications which, on more detailed examination, did not fully satisfy the inclusion criteria.

Data entry

Data were entered into a study database and into an associated RR database. The study-specific information recorded was: Study name; country; region (North America, Europe, Japan or multiple); study design (cohort, nested case-control, or randomized controlled), study population (international, national, regional or specific, *e.g.* workers in a particular industry); study size (number of cases of the disease); year of start; length of follow-up; sexes considered (males only, females only, or both); and age range considered. Also recorded was a summary of the definition of each disease used in each study, including the international classification of disease (ICD) codes where they were provided in the source paper.

The information recorded relating to each RR was: The RR itself and its 95% confidence interval (CI), the RR and CI being estimated from the data provided if necessary; the study to which it related; an identifier for the paper providing the estimate; the year of publication of the paper; whether the RR related to exclusive use of the product; the sex to which it related (males, females or combined - combined RRs only being entered if sex-specific RRs were not available); the age range considered; the years of follow-up considered; the endpoint (from death certification only, or involving in-life diagnosis); whether a latency rule was applied (*i.e.* whether cases identified in the first few years of follow-up were ignored), the number of adjustment factors applied to the risk estimate, and whether the definition of disease was standard or not.

Meta-analyses

Meta-analyses could not be conducted for current cigar or current pipe smoking as the data proved to be too limited. Otherwise, individual study RR estimates were combined using fixed- and random-effects meta-analyses[29], with the

significance of between-study heterogeneity also estimated. For current cigarette smoking, where data were much more extensive, more detailed meta-analyses were conducted, separately for AMI, IHD and stroke, as described below.

Initially, meta-analyses were conducted based on either two RR estimates from each study, if separate RRs were available for males and females, or on a single estimate if the study reported only combined sex results or results for only one sex. Where there was a choice of RRs available for a study, those selected were based on a sequence of preferences applied in turn: (1) Exclusive rather than non-exclusive cigarette smoking; (2) a latency rule had been applied rather than not; and (3) adjustment for the most possible confounders.

Where the data permitted, heterogeneity was studied by the following factors: Sex; region; study population; year of start; study size; exclusive use; study design; lowest age considered; years of follow-up; endpoint; number of adjustment factors; and disease definition. Grouped levels of the variables were used as appropriate.

For each disease, forest plots were generated, with results separated by region, each line of the plot showing the study name (and sex where relevant) and giving the RR and 95%CI. Each RR is illustrated as a square with the area proportional to the weight of the estimate, surrounded by lines extending to the upper and lower 95% confidence limit. The plots also similarly present the overall RRs and 95%CIs for each region and for all the regions combined.

While these meta-analyses and heterogeneity investigations were based on between-study variation in RRs, some additional investigations were conducted on within-study variation in RRs, based on data from the same publication. For sex, these meta-analyses were based on the ratio of the RR for males to that for females, while for level of adjustment, results were compared based on the ratio of the RR adjusted for multiple potential confounding variables to the RR adjusted for no variables. Where multiple pairs of results were available within a publication, the pair selected was chosen based on the preferences described above.

Additional investigation of risk related to the number of cigarettes smoked. The papers selected for the meta-analyses relating cigarette smoking to risk of AMI, IHD and stroke were examined to identify those reporting RRs by number of cigarettes smoked. The results were then tabulated in order to assess those showing a tendency for RRs to increase with amount smoked. Formal meta-analyses of these results were not attempted in view of the various different ways in which the number of cigarettes smoked were grouped. Results by pack-years were not considered as this measure makes the invalid assumption that given increases in amount smoked and duration smoked have the same proportional effect on risk.

RESULTS

Literature searches

A flowchart of the searches is shown in Figure 1. Starting with 20,500 papers identified in the initial MEDLINE searches, the 49 papers identified provided results for AMI, IHD and stroke from respectively, 10, 23 and 31 studies (Figure 1).

For AMI, 20 RRs were available for analysis, all for cigarette smoking. For IHD, there were 53, 51 for cigarette smoking and one each for cigar and for pipe smoking. For stroke there were 76, 70 for cigarettes, four for cigars, and two for pipes. It should be noted that some studies provide more than one estimate, *e.g.* by sex, by level of covariate adjustment, or for different products.

Table 1 (AMI), Table 2 (IHD) and Table 3 (stroke) provide details of the studies considered. Some studies gave data for more than one disease.

The definitions of the diseases considered in each study are not shown in the tables, but can be found in Supplementary material 1.

AMI - cigarette smoking data available

Each study gave data for current cigarette smoking, with the data deriving from one publication per study. Of the total of ten studies, two were from North America [one United States of America (USA), one Canada], and eight were from Europe [two each from Sweden and United Kingdom (UK), and one from each of Estonia, Finland, Germany and Norway]. All were cohort studies. Three studies were national, six regional and one based on GP records. As can be seen in Table 1, the studies varied as regards different factors, including start year, length of follow-up, ages and sexes considered, numbers of AMI cases studied, whether cases were dead or diagnosed, and extent of adjustment for potential confounding factors. As shown in Supplementary material 1, the studies also varied in the definition of AMI, the standard definition being based on ICD-8 or ICD-9 code 410 or ICD-10 code I21.

AMI - cigarette smoking meta-analyses

Data were entered on 20 RRs, with at most four per study. The initial meta-analyses involved 13 RRs, these being selected using the preferences described above. As can be seen in Table 4 and Figure 2, the overall RR estimate (random-effects) was 2.72 (95%CI: 2.40-3.08), this being based on RR estimates that were extremely ($P < 0.001$) heterogeneous, though all exceeded 1.00 (range 1.47-4.72) and all but one of the RRs were significantly increased ($P < 0.05$).

Table 4 also shows RRs by level of ten different study or RR characteristics. The the most striking evidence of risk variation was for number of adjustment factors where the estimates adjusted for age only (2.52, 95%CI: 2.34-2.71) and for age and other factors (2.89, 95%CI: 2.48-3.37) were higher than that with no adjustment (1.47, 95%CI: 1.08-2.01). Estimates were also significantly higher for estimates from North America rather than Europe, for studies starting from 1988 onward than for earlier starting studies, for studies with shorter years of follow-up, and for studies using a standard disease definition. The RR for females exceeded that for males, but not significantly.

Table 1 Details of the 10 studies of acute myocardial infarction

Study ID[a]	Ref.	Country	Design	Study population	Start year	Yr followed	Age	Sex[b]	Cases	Adjust[c]	Excl[d]	Latency[e]	Endpoint	NRR[f]
BIOBANK	[37]	United Kingdom	Cohort	National	2006	12	40-69	M, F	5081	2	0	0	Diagnosed	2
CaCHS	[38]	Canada	Cohort	Regional	2001	13	20+	M, F	1133	15	0	0	Diagnosed	2
CALIBER	[39]	United Kingdom	Cohort	GP records	1997	13	30+	F	5628	1	0	0	Diagnosed	1
EPIC-GERM	[40]	Germany	Cohort	Regional	1994	14	35-65	C	507	0, 9	0	0	Diagnosed	2
ESTONGENOME	[41]	Estonia	Cohort	National	2002	13	18+	M, F	118	0, 1	0	0	Died	4[g]
KIHD	[42]	Finland	Cohort	Regional	1984	18	42-60	M	205	0	0	0	Diagnosed	1
TROMSO	[43]	Norway	Cohort	Regional	1979	33	20-94	F	854	0, 4	0	0	Diagnosed	2
VASTERBOTTEN	[44]	Sweden	Cohort	Regional	1990	19	30-60	C	2062	2, 9	0	0	Diagnosed	2
WHILA	[45]	Sweden	Cohort	Regional	1995	20	50-59	F	205	1, 7	0	0	Diagnosed	2
WHS	[46]	United States	Cohort	National	1992	26	45+	F	629	0, 14	0	0	Diagnosed	2

[a]Study IDs are BIOBANK: The UK Biobank Study; CaCHS: Canadian Community Health Survey; CALIBER: Cardiovascular disease research using linked bespoke studies and electronic health records; EPIC-GERM: European Prospective Investigation into Cancer and Nutrition, German component; ESTON-GENOME: Estonian Genome Center of the University of Tartu; KIHD: Kuopio Ischemic Heart Disease Risk Factor Study; TROMSO: Tromsø Study; VASTERBOTTEN: Västerbotten Intervention Programme; WHILA: Women's Health in the Lund Area Study; WHS: Women's Health Study.
[b]C: Results only for sexes combined.
[c]Number of adjustment factors for which relative risk (RR) available (0: Unadjusted, 1: Age adjusted, N > 1: Adjusted for N factors).
[d]No study had results available for exclusive use.
[e]No study excluded deaths in the early period of follow-up.
[f]Number of RRs available.
[g]Some of the RRs used from this study came from personal communication from Professor Koks.

AMI - cigarette smoking within-study comparisons

There were three comparable pairs of sex-specific RRs from the same study (see Supplementary material 1). The male RR was less than the female one in two pairs, and the overall estimate of the male/female ratio was not significant (ratio 0.74, CI 0.50-1.09).

There were four studies where comparison could be made between estimates adjusted for 2 or more covariates and estimates that were unadjusted or adjusted for age only. In only one of these did adjustment for multiple covariates materially increase the RR.

Within the studies considered, no study has pairs of estimates varying by other factors.

Table 2 Details of the 23 studies of ischaemic heart disease

Study ID[a]	Ref.	Country	Design	Study population	Start year	Yr followed	Age[b]	Sex[c]	Cases[d]	Adjust[e]	Excl[f]	Latency[g]	Endpoint	NRR[h]
7CNTRY-ITALY	[47]	Italy	Cohort	Regional	1960	50	40-59	M	319	3	0	0	Died	1
ARIC	[48]	United States	Cohort	National	1987	30	45-64	C	1798	0, 15	0	0	Diagnosed	2
BIOBANK	[49]	United Kingdom	Cohort	National	2006	12	40-69	C	547	0	0	0	Diagnosed	1
CALIBER	[39]	United Kingdom	Cohort	GP records	1997	13	30+	F	16800	1	0	0	Diagnosed	1
CPS-II	[50]	United States	Cohort	National	1982	22	30+	C	13478	0, 23	0	0	Died	2
CoCHS	[51]	Denmark	Cohort	Regional	1991	22	20-93	F	900	1	0	0	Diagnosed	1
ELSA	[52]	United Kingdom	Cohort	National	2004	13	52+	C	352	0, 7	0	0	Diagnosed	2
EPIC-10	[53]	Multi	Cohort	International	1991	19	35-70	M, F	7198	0	0	0	Diagnosed	2
	[54]	Multi	Nested CC	International	1991	19	35-70	C	7198	0	0	0	Diagnosed	1
EPIC-UK	[55]	United Kingdom	Cohort	Regional	1993	14	45-79	C	2332	0, 2, 6	0	0	Diagnosed	3
ESTON-GENOME	[41]	Estonia	Cohort	National	2002	13	18+	M, F	696	0, 1	0	0	Died	4[i]
FINRISK	[56]	Finland	Cohort	National	1982	25	25-74	F	NR	3, 8	0	0	Died	2
HAPIEE	[57]	Multi	Cohort	International	2002	9	NAR	C	225	0	0	0	Died	1
HSE-SHS	[58]	United Kingdom	Cohort	National	1994	17	NAR	C	1412	0, 7	0	0	Died	2
JACC	[59]	Japan	Cohort	Regional	1988	21	40-79	M, F	1554	0, 7, 9	x	0	Died	4
MALMO	[60]	Sweden	Cohort	Regional	1991	22	46-67	M, F	3217	0, 6	0	0	Diagnosed	4
MESA	[61]	United States	Cohort	Regional	2000	11	45-84	C	449	1, 14	0	0	Diagnosed	3
	[62]	United States	Cohort	Regional	2000	11	45-84	C	449	0	0	0	Diagnosed	1
NAS	[63]	United States	Cohort	Regional	1991	20	NAR	F	137	0	0	0	Diagnosed	1
NHS	[64]	United States	Cohort	Medical workers	1989	17	43-68	F	3874	0	0	0	Diagnosed	1
NHS-II	[65]	United States	Cohort	Medical workers	1991	20	25-42	F	456	1, 15	0	0	Diagnosed	2
PREVEND	[66]	Netherlands	Cohort	Regional	2001	9	32-80	C	212	0, 2, 10	0	0	Diagnosed	3
USA5	[67]	United States	Cohort	Regional	2000	11	55+	M, F	29931	0, 5	0	0	Died	4
WHI	[68]	United States	Cohort	National	1993	20	50-79	F	2975	11	0	0	Died	1
WHITEHALL	[69]	United Kingdom	Cohort	Civil servants	1967	43	40-69	M	3250	1	0	0	Died	1

[a]Study IDs are 7CNTRY-ITALY: Italian Rural Areas of the Seven Countries Study; ARIC: Atherosclerosis Risk in Communities Study; BIOBANK: The UK Biobank Study; CALIBER: Cardiovascular disease research using linked bespoke studies and electronic health records; CPS-II: Cancer Prevention Study 2; CoCHS: The Copenhagen City Heart Study; ELSA: The English Longitudinal Study of Ageing; EPIC-10: European Prospective Investigation Into Cancer and Nutrition; EPIC-UK: The European Prospective Investigation of Cancer -Norfolk; ESTON-GENOME: Estonian Genome Center of the University of Tartu; FINRISK: The National FINRISK Study; HAPIEE: Health, Alcohol and Psychosocial Factors in Eastern Europe (HAPIEE) project; HSE-SHS: Health Survey for England and the Scottish Health Survey; JACC: Japanese Collaborative Cohort Study; MALMO: Malmö Diet and Cancer Study; MESA: Multi-Ethnic Study of Atherosclerosis; NAS: Normative Aging Study; NHS: Nurses' Health Study I; NHS-II: Nurses' Health Study II; PREVEND: Prevention of Renal and Vascular End-Stage Disease; USA5: Cancer Prevention Study II Nutrition, Nurses' Health Study I Women's Health Initiative cohort, National Institutes of Health–AARP Diet and Health Study, and Health Professionals Follow-up Study; WHI: Women's Health Initiative; WHITEHALL: The Whitehall Study.

[b]NAR: No age restriction specified.

[c]C: Results only for sexes combined.

[d]NR: Not reported.

[e]Number of adjustment factors for which relative risk (RR) available (0 = unadjusted, 1 = age adjusted, N>1 = adjusted for N factors).

[f]x: Results available for exclusive use.

[g]No study excluded deaths in the early period of follow-up.

[h]Number of RRs available.

[i]Some of the RRs used from this study came from personal communication from Professor Koks.

IHD – cigarette smoking data available

Each study gave data for current cigarette smoking, with the data deriving from two publications for one study. Of the total of 23 studies, eight were from the USA, 14 from Europe (six UK, two from more than one country, and one from each of Denmark, Estonia, Finland, Italy, Netherlands, and Sweden), and one from Japan. One was a nested case-control study, the rest being of cohort design. Two studies were international, eight national, nine regional, two of medical workers, one of civil servants and one based on general practitioner records.

As demonstrated in Table 2, the studies varied by various factors, including start year, length of follow-up, ages and sexes considered, numbers of IHD cases studied, whether results were available for exclusive cigarette use, whether cases were dead or diagnosed, and the extent of adjustment for potential confounding factors. As shown in Supplementary material 1, the studies also varied with the definition of IHD used to identify cases, the standard definition being based on ICD-8 or ICD-9 codes 410-414 or ICD-10 codes I20-I25.

IHD - cigarette smoking meta-analyses

Data were entered on 49 RRs, with at most four per study. The initial meta-analyses involved 28 RRs, these being selected using the preferences described above. As can be seen in Table 5 and Figure 3, the overall RR estimate (random-effects) was 2.01 (95%CI: 1.84-2.21), this being based on RR estimates that were extremely ($P < 0.001$) heterogeneous, though all but one exceeded 1.00 (range 0.81-4.30), and 27 were significantly increased ($P < 0.05$).

Table 5 also shows RRs by level of 11 different study or RR characteristics. There was significant ($P < 0.05$) variation for two of these. One was endpoint, where the RR was higher for cases that had died compared to where it had been diagnosed. The other related to the number of adjustment factors where the RR was lower for those adjusted for age only, than for those that were unadjusted or adjusted for multiple factors. As for AMI, the RR for females exceeded that for males, but not significantly.

IHD – cigarette smoking within-study comparisons

There were five comparable pairs of sex-specific RRs from the same study (see Supplementary material 1). The male RR was lower in all five pairs, and the overall estimate of the male/female ratio was significant (ratio 0.85, 95%CI: 0.80-0.91).

Table 3 Details of the 31 studies of stroke

Study ID[a]	Ref.	Country	Design	Study population	Start year	Yr followed	Age[b]	Sex[c]	Cases	Adjust[d]	Excl[e]	Latency[f]	Endpoint	NRR[g]
7CNTRY-ITALY	[47]	Italy	Cohort	Regional	1960	50	40-59	M	225	3	0	0	Died	1
ARIC	[48]	USA	Cohort	National	1987	30	45-64	C	1106	0, 14	0	0	Diagnosed	2
BIOBANK	[70]	UK	Cohort	National	2006	12	40-69	M, F	4662	2	0	0	Diagnosed	2
CALIBER	[39]	UK	Cohort	GP records	1997	13	30+	F	11842	1	0	0	Diagnosed	1
CPS-II	[50]	USA	Cohort	National	1982	22	30+	C	5582	0, 23	0	0	Died	2
CaCHS	[38]	Canada	Cohort	Regional	2001	13	20+	M, F	1636	15	0	0	Diagnosed	2
	[71]	Canada	Cohort	Regional	2001	11	20+	M, F	1636	0	0	0	Diagnosed	2
ELSA	[52]	UK	Cohort	National	2004	13	52+	C	326	0, 7	0	0	Diagnosed	2
EPIC-10	[54]	Multi	Nested CC	International	1991	19	35-70	C	2187	0	0	0	Diagnosed	1
EPIC-ITALY	[72]	Italy	Cohort	Regional	1993	15	35-74	M, F	386	0, 2, 10	0	0	Diagnosed	6
EPIC-SPAIN	[73]	Spain	Cohort	Regional	1992	16	29-69	F	301	0	0	0	Diagnosed	1
EPIC-UK	[55]	UK	Cohort	Regional	1993	14	45-79	C	385	0, 2, 6	0	0	Diagnosed	3
ESTON-GENOME	[41]	Estonia	Cohort	National	2002	13	18+	M, F	156	0, 1	0	0	Died	4[h]
HAPIEE	[57]	Multi	Cohort	International	2002	9	NAR	C	109	0	0	0	Died	1
HSE-SHS	[58]	UK	Cohort	National	1994	17	NAR	C	690	0, 7	0	0	Died	2
JACC	[59]	Japan	Cohort	Regional	1988	21	40-79	M, F	3163	0, 7, 9	x	0	Died	4
JHS	[74]	USA	Cohort	Regional	2000	15	21-84	C	183	0, 11	0	0	Diagnosed	2
JP8	[75]	Japan	Cohort	National	1983	30	40+	M	3487	0	0	0	Died	1
MALMO	[76]	Sweden	Cohort	Regional	1991	22	46-67	C	305	0	0	0	Diagnosed	1
MESA	[62]	United States	Cohort	Regional	2000	11	45-84	C	180	0	0	0	Diagnosed	1
MILLION	[28]	United Kingdom	Cohort	National	1996	19	46-66	F	8103	8	0	0	Diagnosed	1
NFBC	[77]	Finland	Cohort	Regional	1966	49	14-46	C	352	0, 10	0	0	Diagnosed	2
NHIS	[78]	United States	Cohort	National	1987	24	18-95	C	2046	0, 5	x	0	Died	2
	[79]	United States	Cohort	National	1987	14	18-95	C	2046	0, 8, 9	0	x	Died	3
	[80]	United States	Cohort	National	1987	28	40-79	M	2046	0, 1, 9	0	x	Died	3

NHS	[64]	United States	Cohort	Medical workers	1989	17	43-68	F	3288	0	0	0	Diagnosed	1
NIH-AARP	[81]	United States	Cohort	Regional	2004	7	70+	C	1369	0, 4	0	0	Died	2
NLMS	[82]	United States	Cohort	National	1985	26	35-80	C	3083	0, 1, 5	x	0	Died	3
OHASAMA	[83]	Japan	Cohort	Regional	1998	12	60+	C	293	2	x	0	Diagnosed	1
PREVEND	[66]	Netherlands	Cohort	Regional	2001	9	32-80	C	83	0, 2, 10	0	0	Diagnosed	3
SCCS	[84]	United States	Cohort	Regional	2002	11	40-79	C	389	7	0	0	Died	1
USA5	[67]	United States	Cohort	Regional	2000	11	55+	M, F	9821	0, 5	0	0	Died	4
WHITEHALL	[69]	United Kingdom	Cohort	Civil servants	1967	43	40-69	M	1061	1	0	0	Diagnosed	1
WHS	[46]	United States	Cohort	National	1992	26	45+	F	887	0, 14	0	0	Diagnosed	2

[a]Study IDs are 7CNTRY-ITALY: Italian Rural Areas of the Seven Countries Study; ARIC: Atherosclerosis Risk in Communities Study; BIOBANK: The UK Biobank Study; CALIBER: cardiovascular disease research using linked bespoke studies and electronic health records; CPS-II: Cancer Prevention Study 2; CaCHS: Canadian Community Health Survey; ELSA: The English Longitudinal Study of Ageing; EPIC-10: European Prospective Investigation into Cancer and Nutrition; EPIC-ITALY: Italian European Investigation into Cancer and Nutrition; EPIC-SPAIN: Spanish European Investigation into Cancer and Nutrition; EPIC-UK: The European Prospective Investigation of Cancer -Norfolk; ESTON-GENOME: Estonian Genome Center of the University of Tartu; HAPIEE: Health, Alcohol and Psychosocial Factors in Eastern Europe (HAPIEE) project; HSE-SHS: Health Survey for England and the Scottish Health Survey; JACC: Japanese Collaborative Cohort Study; JHS: Jackson Heart Study; JP8: Pooled analysis of eight prospective studies in Japan; MALMO: Malmö Diet and Cancer Study; MESA: Multi-Ethnic Study of Atherosclerosis; MILLION: Million Women Study; NFBC: Northern Finland Birth Cohort; NHIS: National Health Interview Survey; NHS: Nurses' Health Study; NIH-AARP: National Institutes of Health–AARP Diet and Health Study; NLMS: National Longitudinal Mortality Study; OHASAMA: The Ohasama Study; PREVEND: Prevention of Renal and Vascular End-Stage Disease; SCCS: Southern Community Cohort Study; USA5: Cancer Prevention Study II Nutrition, Nurses' Health Study I, Women's Health Initiative cohort, National Institutes of Health–AARP Diet and Health Study, and Health Professionals Follow-up Study; WHITEHALL: The Whitehall Study, and WHS: Women's Health Study.

[b]NAR: No age restriction specified.

[c]C: Results only for sexes combined.

[d]Number of adjustment factors for which relative risk (RR) available (0 = unadjusted, 1 = age adjusted, N>1 = adjusted for N factors).

[e]x: Results available for exclusive use.

[f]x: Results available with deaths excluded in early period of follow-up.

[g]Number of RRs available.

[h]Some of the RRs used from this study came from personal communication from Professor Koks.

There were 14 study/sex combinations where comparison could be made between estimates adjusted for two or more covariates and estimates that were unadjusted or adjusted for age only. In all but two of the 14, adjustment for multiple covariates increased the RR ($P < 0.05$).

Within the studies considered, no study has pairs of estimates varying by other factors.

Stroke - cigarette smoking data available

Each study gave data for current cigarette smoking, with the data deriving from three publications for one of these studies, and from two for another. Of the 31 studies, 12 were from North America (11 from USA, one from Canada), 16 from Europe (seven UK, two Italy, two from multiple countries, and one each from Estonia, Finland, Netherlands, Spain and Sweden), and three from Japan. One was a nested case-control study, the rest being of cohort design. Two studies were international, 11 national, 15 regional, one of medical workers, one of civil servants and one based on general practitioner records.

Table 4 Acute myocardial infarction and current *vs* never cigarette smoking – results from random effects meta-analyses

Full output table	Factor	Level	No. of RRs	No. of studies	RR (95%CI)	Test of heterogeneity by level (NS = $P \geq 0.1$) and trend if relevant, *P* value
	All		13	10	2.72 (2.40-3.08)	< 0.001
5	Sex	Combined	2	2	2.98 (2.20-4.04)	
		Males	4	4	2.30 (1.57-3.37)	
		Females	7	7	2.83 (2.40-3.34)	NS
6	Region	N. America	3	2	3.42 (2.93-3.99)	
		Europe	10	8	2.54 (2.22-2.90)	< 0.01
7	Study population	National	5	3	2.85 (2.16-3.77)	
		Regional	7	6	2.69 (2.18-3.33)	
		Other	1	1	2.51 (2.33-2.71)	NS
8	Year of start of baseline	< 1988	2	2	1.81 (1.28-2.56)	
		1988+	11	8	2.93 (2.58-3.32)	< 0.05
9	Number of cases	< 1000	7	6	2.52 (1.96-3.25)	
		1000+	6	4	2.87 (2.46-3.35)	NS
10	Lowest age considered	< 30	5	3	3.02 (2.09-4.35)	
		30-44	6	5	2.58 (2.20-3.04)	
		45+	2	2	2.88 (2.40-3.46)	NS trend NS
11	Yr of follow-up	10-< 20	10	7	2.78 (2.40-3.23)	
		20-< 30	2	2	2.88 (2.40-3.46)	
		30+	1	1	2.11 (1.81-2.46)	< 0.05 trend < 0.01
12	Endpoint	Died	2	1	2.99 (1.34-6.67)	
		Diagnosed	11	9	2.71 (2.38-3.08)	NS
13	Number of adjustment factors	None	1	1	1.47 (1.08-2.01)	
		Age only	3	2	2.52 (2.34-2.71)	
		More	9	7	2.89 (2.48-3.37)	< 0.001
14	Disease definition standard	No	6	7	2.43 (2.06-2.87)	
		Yes	7	5	3.14 (2.63-3.74)	< 0.05

As can be seen in Table 3, the studies varied as regards different factors, including start year, length of follow-up, ages and sexes considered, numbers of stroke cases studied, whether results were available for exclusive cigarette use, or for cases being excluded during the early period of follow-up, whether cases were dead or diagnosed, and the extent of adjustment for potential confounding factors. As shown in Supplementary material 1, the studies also varied with the definition of stroke used to identify cases, the standard definition being based on ICD-8 or ICD-9 codes 430-438 or ICD-10 codes I60-I69.

Stroke - cigarette smoking meta-analyses

Data were entered on 70 RRs, with at most six per study. The initial meta-analyses involved 37 RRs, these being selected using the preferences described above. As can be seen in Table 6 and Figure 4, the overall RR estimate (random-effects) was 1.62 (95%CI: 1.48-1.77), this being based on RR estimates that were extremely ($P < 0.001$) heterogeneous, though all but one of the 37 RRs exceeded 1.00 (range 0.66-2.91), and 32 were significantly increased ($P < 0.05$).

Table 6 also shows RRs by level of 11 different study or RR characteristics, there being highly significant evidence ($P < 0.001$) of variation for three of them. One related to the RR being higher for studies in North America and Europe than for studies in Japan, one to the RR being higher for non-exclusive cigarette smokers than it was for exclusive cigarette smokers, and one to the RR being higher for studies with a shorter follow-up period.

Table 5 Ischaemic heart disease and current *vs* never cigarette smoking – results from random effects meta-analyses

Full output table	Factor	Level	No. of RRs	No. of studies	RR (95%CI)	Test of heterogeneity by level (NS = $P \geq 0.1$) and trend if relevant, *P* value
	All		28	23	2.01 (1.84-2.21)	< 0.001
19	Sex	Combined	10	10	1.94 (1.71-2.21)	
		Males	7	7	1.86 (1.53-2.26)	
		Females	11	11	2.23 (1.86-2.69)	NS
20	Region	N. America	9	8	2.23 (1.92-2.58)	
		Europe	17	14	1.90 (1.67-2.15)	
		Japan	2	1	2.15 (1.73-2.69)	NS
21	Study population	National	9	8	2.10 (1.92-2.30)	
		Regional	12	9	1.85 (1.56-2.19)	
		Other	7	6	2.18 (1.77-2.69)	NS
22	Year of start of baseline	< 1988	5	5	1.89 (1.56-2.27)	
		1988+	23	18	2.04 (1.83-2.28)	NS
23	Number of cases	< 1000	12	11	1.97 (1.58-2.45)	
		1000+	16	12	2.04 (1.83-2.27)	NS
24	Exclusive cigarettes	No	26	22	2.00 (1.82-2.21)	
		Yes	2	1	2.15 (1.73-2.69)	NS
25, 26	Lowest age considered	< 30	5	4	2.45 (1.77-3.39)	
		30-44	11	9	1.81 (1.61-2.05)	
		45+	9	7	2.06 (1.76-2.42)	NS trend without missing NS
		Missing	3	3	2.09 (1.16-3.78)	
27	Yr of follow-up	< 10	2	2	2.52 (1.07-5.90)	
		10-< 20	13	10	2.04 (1.77-2.35)	
		20-< 30	10	8	1.99 (1.78-2.23)	
		30+	3	3	1.68 (1.23-2.29)	NS trend NS
28	Endpoint	Died	13	10	2.23 (1.94-2.57)	
		Diagnosed	15	13	1.83 (1.62-2.05)	< 0.05
29	Number of adjustment factors	None	6	5	2.11 (1.78-2.50)	
		Age only	5	4	1.64 (1.39-1.93)	
		More	17	14	2.10 (1.88-2.35)	< 0.05
30	Disease definition standard	No	13	12	1.83 (1.59-2.10)	
		Yes	15	11	2.17 (1.93-2.45)	< 0.1

Stroke - cigarette smoking within-study comparisons

There were six comparable pairs of sex-specific RRs from the same study (see Supplementary material 1). The male RR was less than the female one in five of the pairs, and the overall estimate of the male/female ratio was significant (ratio 0.90, 95%CI: 0.82-1.00).

There were 18 study/sex combinations where comparison could be made between estimates adjusted for 2 or more covariates and estimates that were unadjusted or adjusted for age only. In all but one of the 18, adjustment for multiple covariates increased the RR ($P < 0.001$). Within the studies considered, no study has pairs of estimates varying by other factors.

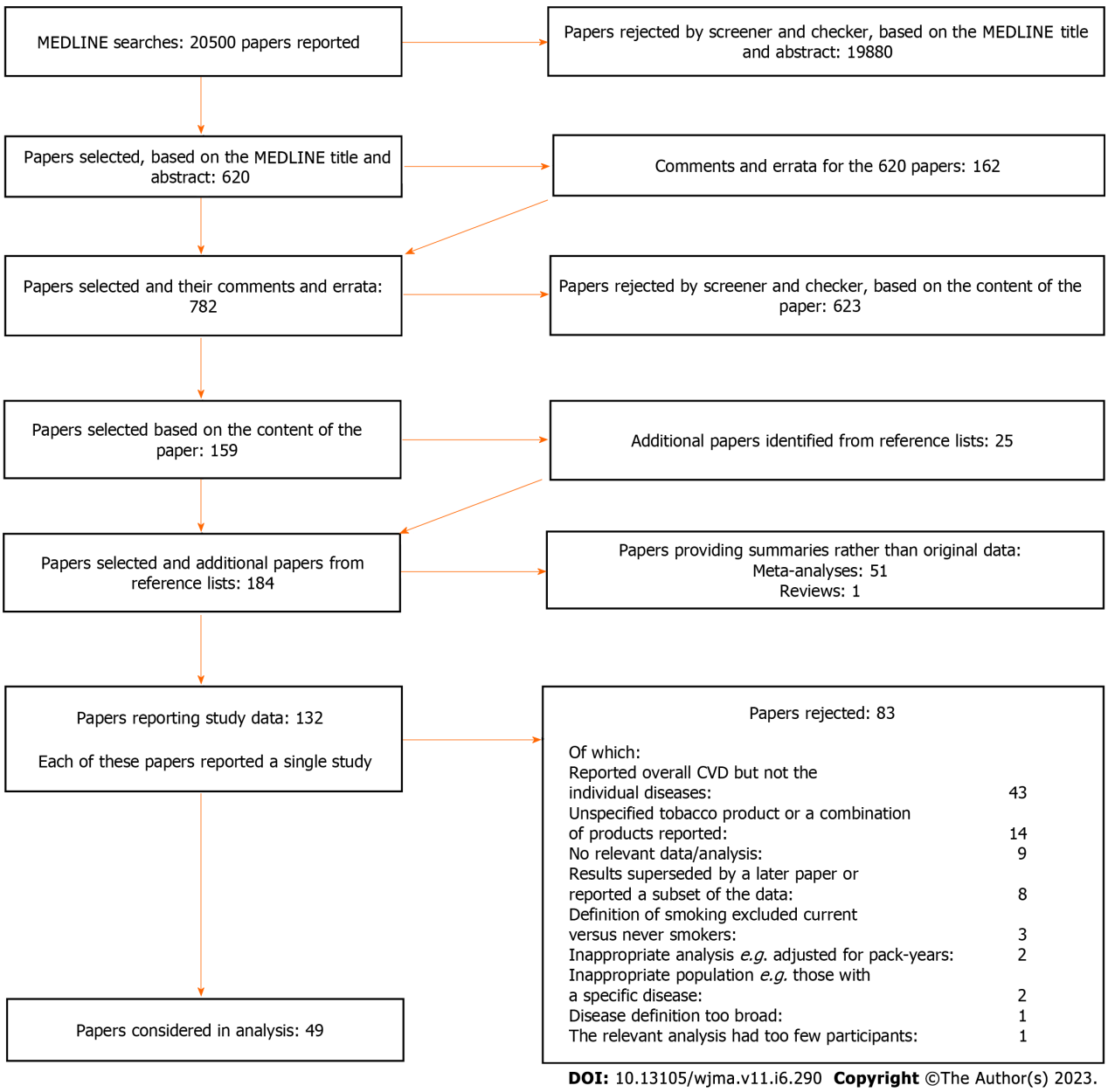
Results relating cigarette smoking to daily amount smoked

The detailed results are given in Supplementary material 3. Fifteen of the studies provided data on RR by amount

Table 6 Stroke and current *vs* never cigarette smoking – results from random effects meta-analyses

Full output table	Factor	Level	No. of RRs	No. of Studies	RR (95%CI)	Test of heterogeneity by level (NS = $P \geq 0.1$) and trend if relevant, *P* value
	All		37	31	1.62 (1.48-1.77)	< 0.001
35	Sex	Combined	17	17	1.65 (1.52-1.50)	
		Males	9	9	1.48 (1.21-1.80)	
		Females	11	11	1.66 (1.39-1.99)	NS
36	Region	N. America	14	12	1.64 (1.48-1.83)	
		Europe	19	16	1.71 (1.51-1.94)	
		Japan	4	3	1.18 (1.04-1.34)	< 0.001
37	Study population	National	13	11	1.76 (1.47-2.11)	
		Regional	19	15	1.55 (1.36-1.78)	
		Other	5	5	1.51 (1.39-1.65)	N.S.
38	Yr of start of baseline	< 1988	8	8	1.43 (1.23-1.67)	
		1988+	29	23	1.68 (1.52-1.85)	< 0.1
39	Number of cases	< 1000	18	16	1.66 (1.44-1.91)	
		1000+	19	15	1.59 (1.41-1.78)	NS
40	Exclusive cigarettes	No	32	27	1.67 (1.52-1.84)	
		Yes	5	4	1.31 (1.19-1.45)	< 0.001
41, 42	Lowest age considered	< 36	8	6	1.59 (1.19-2.14)	
		30-44	16	13	1.48 (1.35-1.62)	
		45+	11	10	1.89 (1.68-2.12)	< 0.01 trend without missing < 0.01
		Missing	2	2	1.87 (1.54-2.28)	
43	Yr of follow-up	< 10	3	3	2.13 (1.80-2.53)	
		10-< 20	22	17	1.69 (1.52-1.89)	
		20-< 30	7	6	1.43 (1.29-1.60)	
		30+	5	5	1.44 (1.10-1.89)	< 0.001 trend < 0.001
44	Endpoint	Died	16	13	1.60 (1.41-1.81)	
		Diagnosed	21	18	1.63 (1.46-1.83)	NS
45	Number of adjustment factors	None	7	7	1.32 (1.08-1.62)	
		Age only	4	3	1.70 (1.31-2.20)	
		More	26	21	1.69 (1.53-1.88)	< 0.1
46	Disease definition standard	No	20	18	1.68 (1.51-1.88)	
		Yes	17	13	1.55 (1.36-1.77)	NS

smoked for one or more of the three diseases, with four giving results for AMI, six for IHD and ten for stroke. Given that some studies presented results separately for females and males, there were a total of 29 independent dose relationships. Twelve of these gave RRs (compared to never smokers) by two levels of amount smoked, and fifteen by three or more levels, with the remaining two dose relationships expressed as risk per daily amount smoked. Fifteen of the relationships came from North American studies, the others coming from European studies. With two minor exceptions (where the stroke results from the ARIC and NHIS studies showed virtually the same RR in heavier smokers as in lighter smokers,) the RR was always greater in the heaviest smoking group than in the lightest smoking group, and in the relationships with three or more levels, the risk increase was usually monotonic. These data demonstrate that a dose-response relationship exists between daily amount smoked and the risk of each of the three diseases.



DOI: 10.13105/wjma.v11.i6.290

Figure 1 Flowchart of the literature searches. CVD: Cardiovascular disease.

Results for cigar and pipe smoking

The detailed output for current smoking of cigars or pipes is given in Supplementary material 2. The data are very limited. There are no data at all for AMI. For IHD the only data come from study MESA, where the RRs compared to never smokers are 0.71 (95%CI: 0.35-1.45) for current smoking of cigars and 0.81 (95%CI: 0.26-4.55) for current smoking of pipes, both estimates being reduced but with very wide 95%CI. For stroke, the available data relates to exclusive product use. For exclusive cigar smoking, an estimate from study NHIS of 1.60 (95%CI: 0.72-3.57) is non-significantly increased, but that from study NLMS of 0.50 (95%CI: 0.21-1.22) is non-significantly reduced. For exclusive pipe smoking, the only study providing data is NLMS, where the RR of 0.24 (95%CI: 0.06-0.91) is significantly reduced.

DISCUSSION

Comparison with earlier reviews - cigarettes

We could find no other meta-analysis published in 2001 to 2020 that related cigarette smoking to the risk of AMI. However, there were various published meta-analyses for the other two diseases, as shown in Table 7 (IHD) and Table 8 (stroke) where their results are summarized and compared with our findings.

For IHD (see Table 7) the nine meta-analyses summarized[5,11,13-19] vary by the year of publication, the regions of the world considered, the definition of what is smoked and the comparison group, and the methodology used. However, the

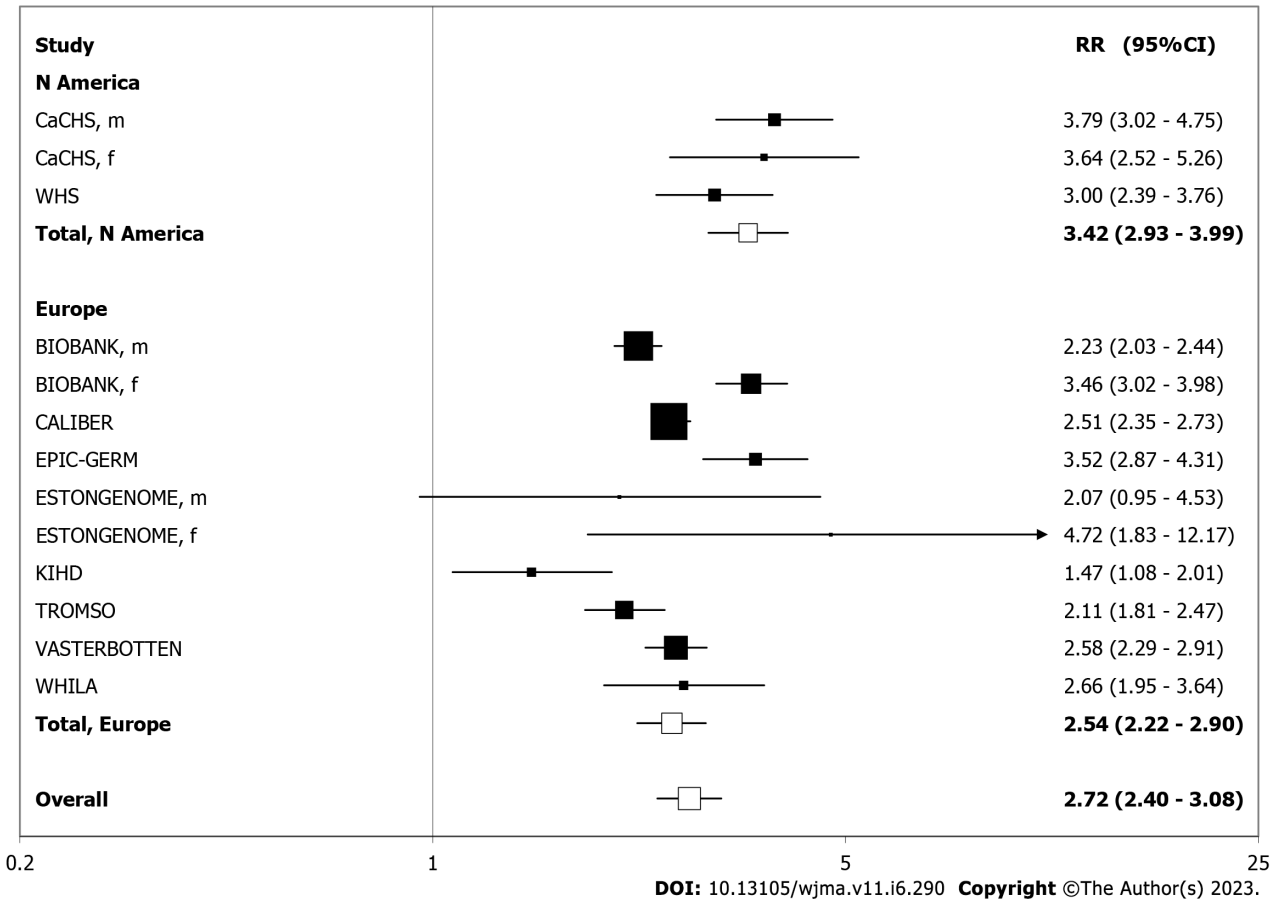


Figure 2 Forest plot for acute myocardial infarction and current *vs* never cigarette smoking, by region.

results are remarkably consistent, with the overall RR estimates varying only from 1.60 to 2.34, as compared with our estimate of 2.01 (95%CI: 1.84-2.21), and all the meta-analyses reporting a somewhat higher RR in females than in males. The consistency of the results, despite the variation in regions considered, also aligns with our finding of similar RRs by continent, though our analysis only included a single study in Japan. Variation in the current smoking RR by any of the factors other than sex or region considered in Table 5 is hardly mentioned at all in any of the earlier meta-analyses. One meta-analysis[11] found no clear relationship, as we did, with study size or number of variables that were adjusted for.

For stroke (see Table 8) data from 11 other meta-analyses[5,11,13-17,20-23] were summarized, these meta-analyses varying by the same factors mentioned above for IHD. Again, the results are quite consistent, with the RRs all significantly raised and varying from 1.32 to 2.27, compared to our estimate of 1.62 (95%CI: 1.48-1.77), and all the meta-analyses reporting a higher RR for females than for males. As previously noted, our analyses found a lower RR for studies in Japan than for studies in North America or Europe (see Table 6), and the earlier results also show relatively low meta-analysis RRs for studies conducted in, or predominantly in, Asia[11,16,17,23]. Few of the earlier meta-analyses considered any of the factors other than sex and region which we had considered in Table 6. One meta-analysis[11] reported higher RRs in studies involving fewer cases, a finding not seen in our analyses (see Table 6) or in another meta-analysis[21]. That meta-analysis reported a non-significantly higher RR in studies with a longer term (> 10 years) follow-up, whereas our analyses reported that the RR declined significantly with increasing follow-up. Our analyses did not consider type of stroke, but a number of the earlier meta-analyses did[17,18,24-28]. It was clear from the RRs reported in these meta-analyses, that the association with smoking was stronger for subarachnoid haemorrhage, where meta-analysis RRs varied from 2.20 to 3.46, than it was for other types of stroke, where RRs varied from 1.19 to 2.17 (data not shown).

For all three diseases our results show strong evidence of a dose-response relationship with amount smoked, a finding consistent with results from earlier meta-analyses (*e.g.*[14]).

Comparison with earlier reviews – cigars and pipes

As noted above, recent data relating to current cigar or pipe smoking are very limited, with no data for AMI, only one study for IHD, and only two for stroke. None of the RRs are significantly increased compared to never smokers, and one, that for stroke and exclusive pipe smoking, 0.24 (95%CI: 0.06-0.91), is significantly reduced. Though there appears to be no recent review for pipe smoking, a recent review[12] reports results from five studies relating current cigar smoking to IHD and from two studies relating current cigar smoking to stroke. From the RRs presented (and using those for primary rather than secondary cigar smoking where both RRs are given for a study) we estimate overall RRs of 1.06 (95%CI: 0.98-1.14) for IHD and 1.00 (0.90-1.11) for stroke, indicating that if any association exists it is much weaker than for cigarettes. It should be noted, however, that all of the RRs cited related to publications in the last century.

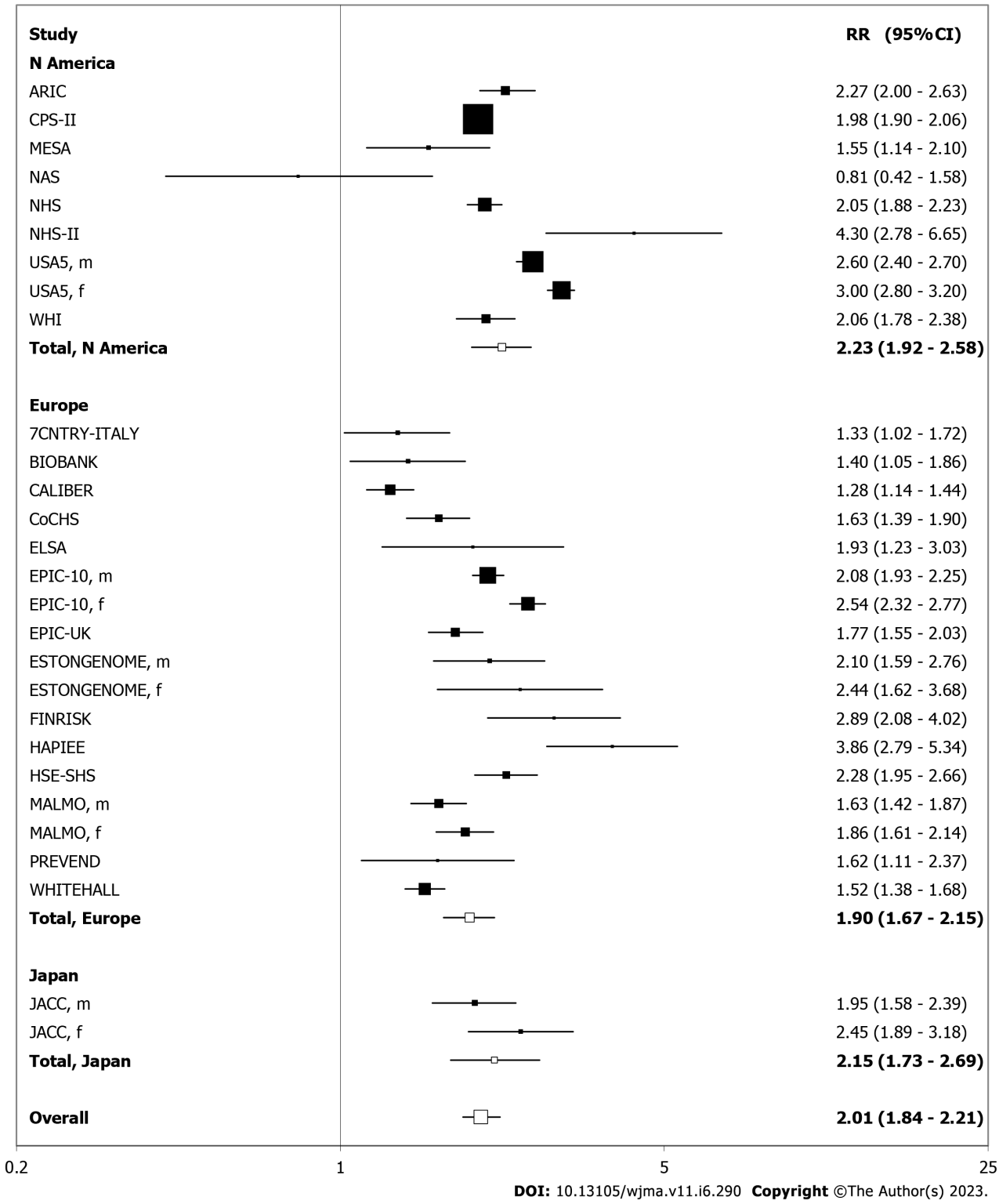
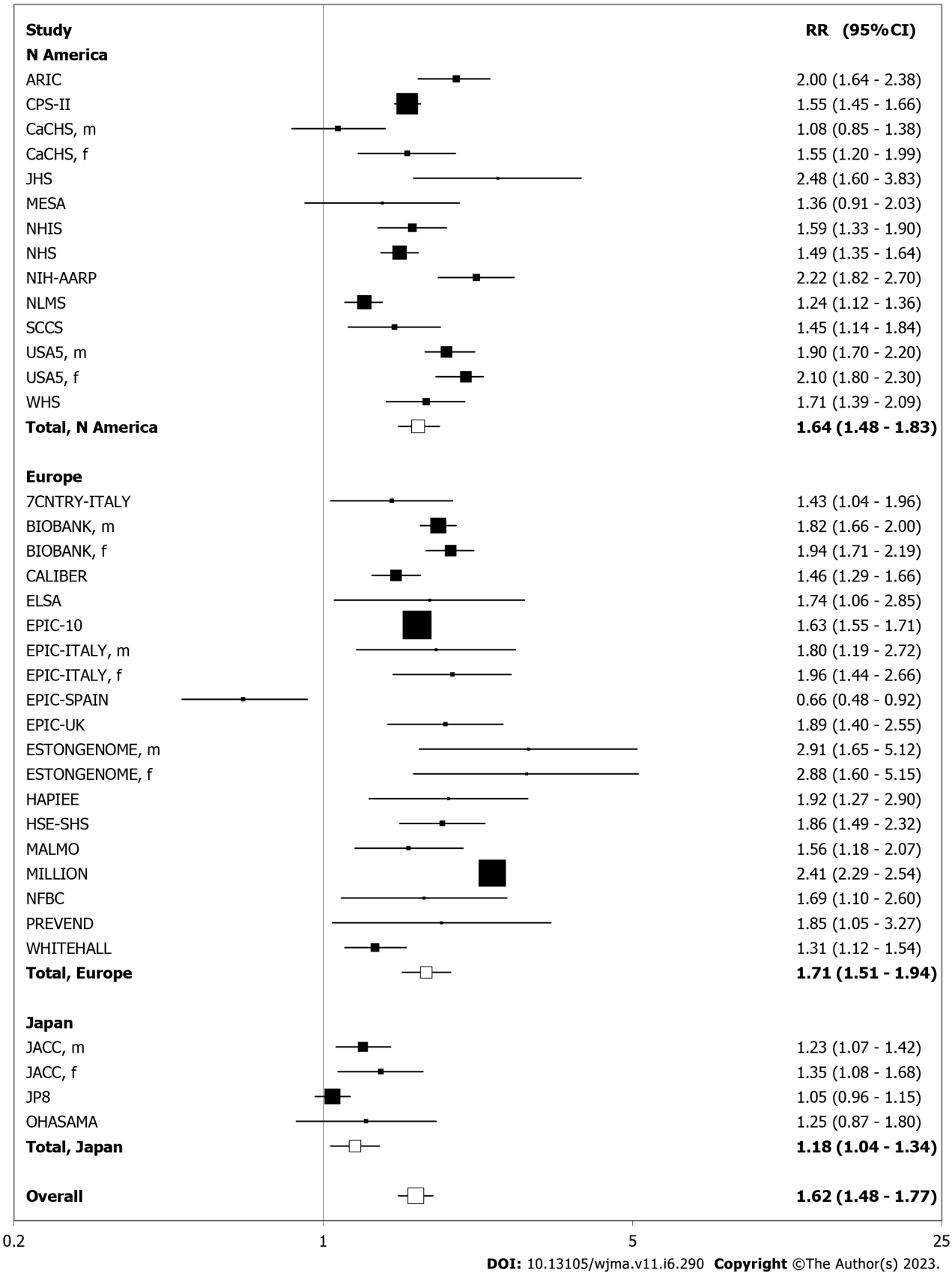


Figure 3 Forest plot for ischaemic heart disease and current *vs* never cigarette smoking, by region.

General considerations

While it is clear that cigarette smoking increases the risk of AMI, IHD and stroke (though by a much smaller factor than for lung cancer and COPD[10]) the RR estimates for all three diseases show highly significant ($P < 0.001$) heterogeneity between the studies. Of the possible reasons for this, many of which are inter-related, we have only investigated some. Thus, populations considered in different studies may vary by race and age, which may affect the product used and extent of exposure. Males and females may also smoke a different amount. The extent of exposure to other risk factors may also vary between studies, as may the extent to which analyses adjust for these factors. As noted previously[10], studies may vary in the definition of exposure, the detail in which changes in smoking over time are monitored or taken into account, the extent to which questions on smoking are answered accurately, the precise definition of disease, and the procedures for diagnosing and treating disease. These factors, not always recorded in the source publications, may help



DOI: 10.13105/wjma.v11.i6.290

Figure 4 Forest plot for stroke and current *vs* never cigarette smoking, by region.

Table 7 Comparison of meta-analysis relative risks for ischaemic heart disease in this study and in other publications

Ref.	Region	What is smoked	Comparison group[a]	RR (95%CI) males	RR (95%CI) females	RR (95%CI) any
Woodward *et al*[17], 2005	Asia-Pacific	Cigarettes	Non	1.56 (1.44-1.70)	1.73 (1.50-2.01)	1.60 (1.49-1.72)
Woodward *et al*[18], 2005	Asia, Australia, New Zealand	Cigarettes	Non			1.86 (1.69-2.06)
Nakamura *et al*[16], 2009	Asia	Undefined	Never			1.97 (1.66-2.33)
Huxley *et al*[19], 2011	Any	Cigarettes	Non	1.72 (1.57-1.88)	1.92 (1.66-2.23)	1.79 (1.61-1.98)
Mons *et al*[15], 2015	Any	Undefined	Never	1.80 (1.51-2.15)	2.26 (1.98-2.59)	2.03 (1.63-2.54)[b]
Lee *et al*[5], 2017[c]	North America, Europe, Asia	Cigarettes[d]	Never	1.99 (1.81-2.19)	2.12 (1.87-2.40)	2.05 (1.90-2.21)
Colpani *et al*[13], 2018	Any	Cigarettes[e]	Never		3.12 (2.15-4.52)	
Hackshaw *et al*[14], 2018	Any	20 cigarettes per day	Never	2.04 (1.86-2.24)	2.84 (2.21-3.64)	2.34 (1.96-2.79)
Lee *et al*[11], 2018[c]	Japan	Cigarettes[d]	Never	1.98 (1.74-2.25)	2.59 (2.06-3.27)	2.21 (1.96-2.50)
This meta-analysis	North America, Europe, Japan	Cigarettes	Never	1.86 (1.53-2.26)	2.23 (1.86-2.69)	2.01 (1.84-2.21)

[a]Former smokers are included among nonsmokers, but are not included among never smokers.
[b]Estimated from data provided.
[c]Includes results for coronary heart disease and acute myocardial infarction.
[d]Includes results for any product if those for cigarettes not available.
[e]Assumed to be cigarettes as study in women.

Table 8 Comparison of meta-analysis relative risks for stroke in this study and in other publications

Ref.	Region	What is smoked	Comparison group[a]	RR (95%CI) males	RR (95%CI) females	RR (95%CI) any
Woodward *et al*[17], 2005	Asia-Pacific	Cigarettes	Non	1.29 (1.20-1.38)	1.42 (1.26-1.62)	1.32 (1.24-1.40)
Nakamura *et al*[16], 2009	Asia	Undefined	Never			1.34 (1.12-1.48)
Peters *et al*[22], 2013	Any	Cigarettes	Non	1.67 (1.49-1.88)	1.83 (1.58-2.12)	1.73 (1.58-1.89)
Chen *et al*[20], 2014	Western	Cigarettes	Never			2.27 (1.76-2.93)
Mons *et al*[15], 2015	Any	Undefined	Never	1.44 (1.23-1.68)	1.78 (1.46-2.17)	1.59 (1.29-1.95)[b]
Lee *et al*[5], 2017	North America, Europe, Asia	Cigarettes[c]	Never	1.42 (1.29-1.56)	1.54 (1.33-1.78)	1.48 (1.37-1.60)
Wang *et al*[23], 2017	China	Undefined	Undefined			1.53 (1.06-2.20)[b]
Colpani *et al*[13], 2018	Any	Cigarettes[d]	Never		2.09 (1.51-2.89)	
Hackshaw *et al*[14], 2018	Any	20 cigarettes per day	Never	1.64 (1.48-1.82)	2.16 (1.69-2.75)	1.90 (1.54-2.35)
Lee *et al*[11], 2018	Japan	Cigarettes[c]	Never	1.32 (1.16-1.51)	1.50 (1.16-1.94)	1.40 (1.25-1.57)
Pan *et al*[21], 2019	Any	Cigarettes[c]	Never[e]	1.54 (1.11-2.13)	1.88 (1.45-2.44)	1.92 (1.49-2.48)
This meta-analysis	North America, Europe, Japan	Cigarettes	Never	1.48 (1.21-1.80)	1.66 (1.39-1.99)	1.62 (1.48-1.77)

[a]Former smokers are included among non smokers, but are not included among never smokers.
[b]Estimated from data provided.
[c]Includes results for any product if those for cigarettes not available.
[d]Assumed to be cigarettes as study in women.

[e]Sex-specific relative risks (RRs) are compared to non-smokers.

to explain variations between studies, and between our results and earlier meta-analyses.

Limitations of our work

Though limited to specific regions, and not providing any information relevant to developing countries, our meta-analyses provide a good idea of the size of the RR for current *vs* never cigarette smoking for all three diseases studied, which was our main objective. Although heterogeneity of the individual RR estimates limits the precision of the overall estimates, we have studied various factors that could contribute in part to the heterogeneity. However, we have not carried out multivariate analyses investigating how RRs vary jointly by the studied factors. For smoking of cigars and pipes, our estimates are limited by the paucity of available information. Our analyses are also limited by the lack of clear description of the factors considered in some studies. Notably, in some studies we cannot always tell with certainty whether the term "smoking" relates to any tobacco product use, to cigarette smoking or to exclusive cigarette smoking.

Other limitations arose as the objectives of our study were limited. Thus we did not consider RRs by duration of smoking, age of starting to smoke or individual types of the product smoked (such as tar level of cigarettes). Nor did we consider RRs for former smokers or users of multiple products, and we carried out only a limited assessment relating to amount smoked. Nor did we study variation by the age when the endpoint was diagnosed or when the subject died from it. Nor did we try to determine the extent of bias arising from misclassification of exposure, disease, or confounding variables.

We did not consider results for different types of stroke, which might have given insight into, for example, whether smoking increases risk differently for lacunar and non-lacunar stroke, a stronger association for lacunar stroke being reported in some studies (*e.g.*[30,31]), but being not clearly evident in others (*e.g.*[32-36]). Clearly there is scope for more detailed investigation.

CONCLUSION

Results from 10 studies of AMI, 23 of IHD and 31 of stroke published in 2015-2020 confirm a dose-related association of current cigarette smoking with all three diseases, with RRs somewhat higher for females than males, and for stroke only, and lower for studies in Japan than for studies in North America and Europe. Very limited evidence for current cigar and current pipe smoking shows no increase in risk for IHD and stroke, no data being available for AMI. Our findings seem generally consistent with data from other reviews and meta-analyses published this century. As noted in our companion paper on lung cancer and COPD, cigarettes smokers should quit to most effectively reduce the risks, though switching to other products containing nicotine, may greatly reduce these risks, as as has been most clearly demonstrated for Swedish snuff ("snus").

ARTICLE HIGHLIGHTS

Research background

While there are considerable data on risks from smoking, such risks may change with time, and recent evidence is required for smoking of cigarettes, cigars and pipes.

Research motivation

To take into account recent data on the risks of acute myocardial infarction (AMI), ischaemic heart disease (IHD) and stroke associated with current smoking of cigarettes, cigars and pipes.

Research objectives

To summarize recent data on the risk of AMI, IHD and stroke related to current cigarette, cigar and pipe smoking in North America, Europe and Japan.

Research methods

Searches on MEDLINE identified publications in English in 2015-2020 giving data on risks of the three diseases associated with current (*vs* never) cigarette, cigar or pipe smoking in studies conducted in the three regions. Studies were accepted which were of cohort or nested case-control design or were randomized controlled trials, which involved at least 100 cases of the disease of interest, and were not restricted to specific disease subsets, to patients with specific medical conditions or which reported results superseded by later reports of the study. Relative risk estimates were extracted from each study and overall estimates derived using random-effects meta-analyses.

Research results

There were available results from 10 studies for AMI, from 23 studies for IHD, and from 31 studies for stroke, the studies

being mainly conducted in North America and Europe. Overall relative risk (RR) estimates for current cigarette smoking were 2.72 for AMI, 2.01 for IHD and 1.62 for stroke. Estimates were dose-related to daily cigarette consumption, and somewhat higher for females than males. Estimates were relatively low in Japan for stroke. RR estimates tended to be higher for studies starting later and with a shorter follow-up period and where adjusted for multiple covariates. Only a few studies in the United States provided findings for current cigar or current pipe smoking, and then only for IHD and stroke. There was no evidence from these studies that smoking either of these products increased risk of these diseases.

Research conclusions

Consistent with evidence from earlier studies, increased risks for all three diseases are clearly seen for current cigarette smoking, but not for current cigar or pipe smoking.

Research perspectives

Cigarette smoking increases the risks of developing AMI, IHD and stroke, though by a factor much lower than for lung cancer and chronic obstructive pulmonary disease. To reduce these risks most effectively, cigarette smokers should quit, though switching to other products containing nicotine, such as Swedish snuff ("snus"), may also materially reduce these risks.

ACKNOWLEDGEMENTS

We thank Yvonne Cooper for secretarial assistance and for obtaining the required references. We also thank Professor Sulev Koks for providing additional information from study ESTONGENOME.

FOOTNOTES

Author contributions: Lee PN planned the study; Literature searches were carried out by Coombs KJ and by Lee PN; Statistical analyses were carried out by Hamling JS and checked by Lee PN; Lee PN drafted the text, which was checked by Coombs KJ and Hamling JS.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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S-Editor: Liu JH
L-Editor: A
P-Editor: Zhao S

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