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EDITORIAL

AMPK-associated signaling to bridge the gap between fuel metabolism and hepatocyte viability

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Abstract

The adenosine monophosphate-activated protein kinase (AMPK) and p70 ribosomal S6 kinase-1 pathway may serve as a key signaling flow that regulates energy metabolism; thus, this pathway becomes an attractive target for the treatment of liver diseases that result from metabolic derangements. In addition, AMPK emerges as a kinase that controls the redox-state and mitochondrial function, whose activity may be modulated by antioxidants. A close link exists between fuel metabolism and mitochondrial biogenesis. The relationship between fuel metabolism and cell survival strongly implies the existence of a shared signaling network, by which hepatocytes respond to challenges of external stimuli. The AMPK pathway may belong to this network. A series of drugs and therapeutic candidates enable hepatocytes to protect mitochondria from radical stress and increase cell viability, which may be associated with the activation of AMPK, liver kinase B1, and other molecules or components. Consequently, the components downstream of AMPK may contribute to stabilizing mitochondrial membrane potential for hepatocyte survival. In this review, we discuss the role of the AMPK pathway in hepatic energy metabolism and hepatocyte viability. This information may help identify ways to prevent and/or treat hepatic diseases caused by the metabolic syndrome. Moreover, clinical drugs and experimental therapeutic candidates that directly or indirectly modulate the AMPK pathway in distinct manners are discussed here with particular emphasis on their effects on fuel metabolism and mitochondrial function.

Key words: Adenosine monophosphate-activated protein kinase; Cell survival; Energy metabolism; Fatty liver; Insulin resistance; Glycogen synthase kinase 3β; p70 ribosomal S6 kinase-1

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INTRODUCTION

Metabolic regulation of carbohydrate, lipid and protein, and synthesis of proteins and lipids are the principal functions of the liver, as well as xenobiotic detoxification. The

function and survival of organisms are dependent on the dynamic control of energy metabolism. The regulation of fuel metabolic processes can be mediated by hormones and other endogenous ligands in response to changes in energy status. Diverse signaling pathways contribute to the regulation of energy metabolism, which is associated with the activation of cell surface and nuclear receptors in hepatocytes. Thus, the modulation of specific pathways can provide therapeutic strategies for hepatic diseases that result from metabolic derangements[1].

In a variety of hepatic diseases, abnormal fat accumulation in the liver is often a prerequisite metabolic event for further pathogenesis[2]. Lipotoxicity can lead to the generation of oxidative stress and inflammation, ultimately causing apoptosis[3]. Programmed cell death is elicited by cell surface death receptors, the caspase cascade, deranged mitochondrial metabolism, and energy deficiency. Mitochondria, cytoplasmic organelles in eukaryotic cells, play a key role in energy utilization such as oxidative phosphorylation; dysfunction of mitochondria is closely related with apoptosis[4].

The relationship between fuel metabolism and cell survival strongly implies the existence of a shared signaling network, which is responsible for the regulation of both phenomena. Emerging evidence indicates that the adenosine monophosphate (AMP)-activated protein kinase (AMPK) and p70 ribosomal S6 kinase-1 (S6K1) pathway serves as a key pathway that regulates fuel energy metabolism. In addition, it has been suggested that AMPK controls the redox-state and mitochondrial function. In this review, we focus on the role of the AMPK pathway in hepatic fuel metabolism in conjunction with cell survival. Moreover, clinical drugs and experimental therapeutic candidates that activate the AMPK-S6K1 pathway in distinct manners are discussed here with particular reference to their roles in mitochondrial function and energy metabolism.

FUEL METABOLISM AND SIGNALING PATHWAYS IN THE LIVER

The liver plays a central role in fuel metabolism, and thus regulates dynamic catabolic and anabolic processes to maintain energy homeostasis of organisms. Breakdown products of carbohydrate and lipid (i.e. glucose and fatty acids) are common energy sources which are converted to adenosine-triphosphate (ATP) in mitochondria. In addition, mitochondria have many other metabolic functions, such as regulation of membrane potential, cellular metabolism, calcium signaling (including calcium-induced apoptosis), and apoptosis. During the process of catabolism, the mitochondrion serves as the main source of energy for the cell because it converts nutrients into energy *via* cellular respiration[5]. Most of the oxygen delivered to cells or organs is consumed by mitochondria for ATP generation. When the energy is excessive in the cell, mitochondrial energy production is inhibited so that glucose and free fatty acids can be stored as glycogen and fat through anabolic processes.

AMPK signaling pathways for fuel metabolism

AMPK: AMPK is a heterotrimer complex that consists of a catalytic subunit (α1/2) and two regulatory subunits (β1/2 and γ1/2/3), and functions as a serine/threonine protein kinase[6]; AMPK activation is mediated by phosphorylation of threonine-172 in the catalytic domain of the α subunit[7]. The activity of AMPK can be regulated by upstream kinases, which include liver kinase B1 (LKB1)[8], Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK) β[9], and transforming growth factor β-activated kinase-1[10]. Both LKB1 and CaMKK increase the AMPK activity through direct phosphorylation of threonine-172 in the α subunit. In addition, LKB1 is constitutively active as a major upstream kinase. The upstream signaling molecules of LKB1 may include protein kinase C (PKC)-ζ[11], protein kinase A[12], and p90 kDa ribosomal S6 kinase[13]. The fact that the calcium/calmodulin complex regulates CaMKK suggests that AMPK may be involved in Ca^{2+} modulation in cells.

AMPK regulates energy homeostasis in various organs through response to hormones and nutrient signals. AMPK physiologically responds to the change in the AMP:ATP ratio, and thus serves as an intracellular sensor for energy homeostasis[7]. In addition to ATP production with switching off from anabolic processes in tissues, the activation of AMPK affects whole body fuel utilization and induces fatty acid oxidation and glucose uptake in skeletal muscle and heart, but inhibits lipogenesis and adipocyte differentiation[6-7]. In the liver, AMPK inhibits gluconeogenesis and synthesis of glycogen, fatty acid and cholesterol. Since AMPK plays a key role in metabolic regulation, it is recognized as an important target for metabolic disorders such as obesity, diabetes, and metabolic liver diseases.

S6K1: S6K1 is a mitogen-activated serine/threonine protein kinase that is associated with growth and cell cycle progression. In translational processes, S6K1 phosphorylates the S6 protein of the 40S ribosomal subunit. Phosphoinositide-3 kinase (PI3K)-the mammalian target of rapamycin (mTOR) regulates S6K1 as a distinct pathway from the Ras/mitogen-activated protein kinase cascade[14]. S6K1 signaling suppresses catabolic events such as lipolysis in adipose tissue and fatty acid oxidation in muscle, both of which stimulate ATP generation[15]. Since S6K1 is sensitive to nutrients including amino acids, nutrients negatively regulate insulin signaling by phosphorylating insulin receptor substrate-1 (IRS1) through S6K1 activation. Thus, S6K1 may also affect the regulation of nutrient and hormone signaling pathways under normal and pathological conditions (e.g. obesity, diabetes, and cancer). Moreover, S6K1 may play a role in the balance between survival and death in tissues including the liver. It is noteworthy that AMPK activation leads to inhibition of the mTOR/S6K1 pathway through tuberous sclerosis protein 2 (TSC2) phosphorylation[16]. The regulation of S6K1 by AMPK is now recognized as an important regulatory step, by which cells maintain energy metabolism.

AMPK as a target for metabolic diseases

Nonalcoholic fatty liver disease (NAFLD) is defined as a common liver disease ranging from steatosis to nonalcoholic steatohepatitis, and cirrhosis[2]. Moreover, NAFLD is considered as a main hepatic component of metabolic syndrome[17]. The characteristics of metabolic syndrome are obesity, insulin resistance, and cardiovascular disorders. In obese people mostly with insulin resistance, excessive fat is deposited in the liver and the raised hepatic lipid amount is closely associated with pathogenic processes of the syndrome[18,19].

Hepatic steatosis by liver X receptor-α-sterol regulatory element, binding protein-1c: A variety of conditions such as excess delivery of fatty acids, decreased oxidation of hepatic fatty acid and/or impaired synthesis or secretion of very low-density lipoprotein increase the sources of hepatic lipids, leading to fatty liver disease. The amount of accumulated fat is also increased by lipogenesis; emerging evidence supports the importance of *de novo* lipogenesis in abnormal hepatic fat accumulation in NAFLD patients[20,21]. Lipogenesis is transcriptionally regulated by the membrane-bound sterol regulatory element, binding protein-1c (SREBP-1c), which belongs to the basic helix-loop-helix-leucine zipper family. In the nucleus, SREBP-1c activates transcription of genes involved in lipogenesis, as supported by the finding that the overexpression of SREBP-1c in transgenic mice promotes the development of fatty liver. In animal models of insulin-resistant diabetes and obesity, the increased synthesis of fatty acids contributes to the development of hepatic steatosis.

Liver X receptor-α (LXRα), a transcriptional nuclear receptor, is a key regulator of lipogenic genes encoding for the enzymes that promote hepatic fat accumulation (e.g. fatty acid synthase, FAS; acetyl-CoA carboxylase, ACC; and stearoyl-CoA desaturase-1, SCD-1)[22,23]. Ligand activation of LXRα promotes induction of the lipogenic genes through SREBP-1c, causing increases in fatty acid synthesis and progression to steatosis, hypertriglyceridemia, and steatohepatitis[22]. Thus, SREBP-1c is an important target gene of LXRα. Since the LXRα-SREBP-1c pathway activates lipogenesis in the liver, it is an attractive target for the treatment of hepatic steatosis and hepatitis. In clinical situations, the expression of SREBP-1c and lipogenic genes including ACC and FAS is enhanced in NAFLD patients[24,25]. In addition, increases in LXRα target gene expression (e.g. ACC and FAS) were observed in the patients with fatty liver, which was accompanied by SREBP-1c activation, but not activation of carbohydrate responsive element-binding protein[26].

The AMPK-S6K1 pathway is involved in the regulation of LXRα-SREBP-1c and thus in LXRα-induced lipogenesis; chemical activation of AMPK in conjunction with its inhibition of S6K1 leads to the intervention of hepatic steatosis (Figure 1)[27]. As an example, AMPK activation by oltipraz treatment inhibits S6K1 activity, which inhibits the activity of LXRα[27] and prevents the ability of activated LXRα to bind the LXR binding site upstream

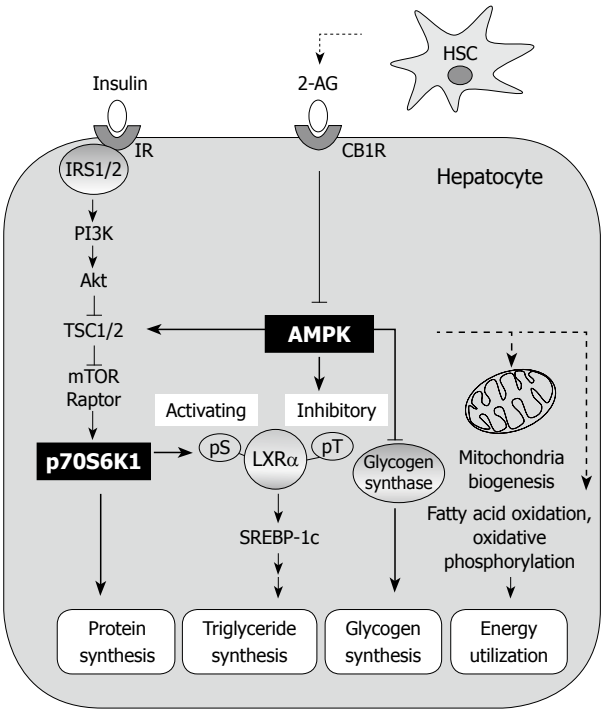

Figure 1 Adenosine monophosphate-activated protein kinase pathway in hepatic fuel metabolism. Adenosine monophosphate-activated protein kinase (AMPK), a metabolic energy sensor, negatively regulates protein synthesis through inhibition of the mammalian target of rapamycin (mTOR)-S6 kinase-1 (S6K1) pathway. The inhibitory effect of AMPK on liver X receptor-α (LXRα)-dependent triglyceride synthesis is opposed by the action of S6K1. AMPK also shuts down glycogen synthesis via inhibitory phosphorylation of glycogen synthase. AMPK as a fuel sensor induces glucose transport and fat oxidation in response to metabolic stress such as energy deprivation, and also increases mitochondrial biogenesis. AMPK counteracts energy depletion by stimulating energy production and limiting energy utilization. Endocannabinoids such as 2-arachidonoylglycerol derived from hepatic stellate cells decrease AMPK phosphorylation resulting in downregulation of lipogenic action. 2-AG: 2-arachidonoylglycerol; CB1R: Cannabinoid 1 receptor; HSC: Hepatic stellate cell; IR: Insulin receptor; Raptor: Regulatory-associated protein of mTOR; IRS1: Insulin receptor substrate-1; PI3K: Phosphoinositide-3 kinase; TSC1: Tuberous sclerosis complex 1; pS: Phospho-serine; pT: Phospho-threonine; SREBP-1c: Sterol regulatory element, binding protein-1c.

of the genes including SREBP-1c and CYP7A1. Therefore, the consequent repression of SREBP-1c expression contributes to decreased synthesis of fat in the liver[27].

Repeated alcohol consumption decreases the production of adiponectin secreted from adipocytes[28]. Adiponectin increases hepatic fatty acid oxidation through AMPK activation[29]. Therefore, it is tempting to speculate that AMPK activity is repressed as hepatic function deteriorates in alcoholic patients. Similarly, AMPK activity was decreased in animals which consumed alcohol for 4 wk[30]. As a compensatory response, alcohol consumption increased lipogenesis in the liver, which may also result from the reduced rate of fatty acid oxidation. Thus, pharmacological activation of AMPK may be of help in treating hepatic steatosis. Peroxisome proliferator-activated receptors (PPARs) play a role in sensing nutrient levels and regulating lipid and glucose metabolism[31]. Thiazolidinediones (TZDs) and fibrates that activate PPARγ and PPARα, respectively, are prescribed for patients with diabetes and/or

dyslipidemia. In those taking PPARγ agonists, insulin-mediated adipose tissue uptake and storage of free fatty acids are augmented with the inhibition of hepatic fatty acid synthesis, which may result in part from indirect activation of AMPK[32,33].

Hepatic insulin resistance: Insulin signaling is important in maintaining homeostasis of glucose, lipid, and protein metabolism, and thus induces anabolism in tissues. In addition, it has effects on normal growth and development. Insulin receptor and its associated protein IRS1 relay signal transmission to the PI3K-Akt pathway, which consequently increases mTOR-S6K activity. Activation of the mTOR-S6K1 pathway by insulin may lead to fat accumulation in adipose tissue, hypertrophy of skeletal muscle, growth of pancreatic β cells, and protein synthesis[15]. Therefore, the control of insulin signaling is tightly regulated by a negative feedback mechanism. In fact, the downstream components of the insulin receptor give inhibitory autoregulatory signals to upstream molecules along the insulin-signaling pathway or through unrelated pathways that cause insulin resistance. In particular, phosphorylation of IRS proteins on serine residues is a key step in the processes of physiological and pathological conditions. So, the kinases that phosphorylate IRS1/2 have been extensively studied.

Hepatic steatosis alone, or to a greater degree in combination with endotoxin challenge, makes the liver susceptible to oxidative damage and thus facilitates the pathologic process of hepatitis. The cytokines produced by accumulated fat with or without endotoxin cause insulin resistance. In particular, tumor necrosis factor α (TNFα) and interleukin-6 (IL-6) lead to insulin resistance through multiple mechanisms. These include c-Jun N-terminal kinase 1 (JNK1)-mediated serine phosphorylation of IRS-1, IκB kinase-dependent nuclear factor-κB activation, and suppressors of cytokine signaling-3 (SOCS-3) induction[34-36]. Since TNFα increases insulin resistance in peripheral organs, inhibition of TNFα activity and/or its decreased expression would be of help to overcome insulin resistance. However, IL-6 displays pleiotropic functions in a tissue-specific and time-dependent manner. IL-6 confers insulin resistance in hepatocytes through activation of SOCS protein through the Jak/Stat pathway to inhibit tyrosine phosphorylation of IRS1[36], while IL-6 increases insulin sensitivity by stimulating basal glucose transport in 3T3-L1 adipocytes[37], smooth muscle[38] and chondrocytes[39]. Acute treatment with IL-6 increases insulin sensitivity due to AMPK activation[40], while chronically elevated IL-6 leads to impaired insulin signaling and cellular insulin resistance *via* activating SOCS-3[36] and reducing the expression of the adiponectin, GLUT4, IRS1 mRNA, IRS-1 protein and its tyrosine phosphorylation[41,42].

Glucose is overproduced in the liver of patients with type 2 diabetes[43]. Because AMPK serves as an energy-saving mechanism, its activation decreases hepatic gluconeogenesis. The experimental results using gene knockouts, pharmacological means, or adenoviral activation of AMPK support the role of AMPK in the regulation of glucose production in the liver. Consistently, hepatic glucose production increased to show hyperglycemia and glucose intolerance in liver-specific AMPKα2 deficient mice. Hence, it is highly likely that the hepatic AMPKα2 isoform is critical for repressing hepatic glucose production and maintaining fasting blood glucose levels in the physiological range[44]. Consistently, AMPK activation by adenovirus expressing a constitutively active form of AMPKα2 as well as by 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR, a direct AMPK activator) or metformin reduced glucose output[45-47].

Activation of S6K1 exerts a negative feedback action on insulin signaling. As an example, TNFα secreted by non-parenchymal cells activates S6K1 in pathologic states. The important role of S6K1 on insulin resistance was proven by a study using S6K1-null mice[48,49]. A key role for mTOR-S6K1 regulation of insulin resistance was also supported by the finding that rapamycin blocked IRS1 phosphorylation[50,51], confirming the importance of S6K1 activity in inducing insulin resistance. Hence, insulin resistance induced by abnormal conditions such as hyperinsulinemia, obesity and excess nutrient availability is accompanied by an increase in S6K1 activity[48,52]. The result of a study using a knockout model proved the critical role of S6K1 and its physiological feedback importance to IRS1/2 and PI3K for insulin resistance. In an experimental model, the inhibitory effect of high-fat diet consumption on the insulin receptor-PI3K pathway is also mediated by S6K1. In our laboratory, it was found that the inhibitory modulation of S6K1 activity by beneficial candidates reversed insulin resistance and hyperglycemia[50]. In particular, oltipraz treatment inhibited S6K1 through AMPK activation. Consistently, a dominant negative mutant of AMPK abrogated S6K1 phosphorylation[50]. AMPK activation by other drugs like metformin and rosiglitazone also contribute to insulin sensitivity enhancement[47,53]. Similarly, other agents that inhibit insulin resistance also antagonize S6K1 activation downstream of AMPK[50]. So, these agents have the effects of improving insulin sensitivity through a mechanism involving AMPK-mediated S6K1 inhibition in hepatocytes[50].

JNK1 is activated by various stress signals such as cytokines or oxidative stress, and thus the activity of JNK1 increases under prediabetic or diabetic conditions. This important kinase is also implicated in the phosphorylation of IRS1/2[54-56], interfering with insulin action. The JNK pathway is stimulated by oxidative stress conditions, increased flux of free fatty acids and TNFα production, which contributes to developing insulin resistance. The importance of JNK activation is supported by the finding that a deficiency of JNK1 prevented insulin resistance in an experimental model[54]. Moreover, JNK mediates dysfunction of insulin secretion from β cells[57]. Hence, inhibition of JNK by chemical means may help improve insulin resistance and ameliorate hepatic energy metabolism[54,58]. For example, isoliquiritigenin from various natural herbs including licorice has a JNK-inhibitory effect. Thus, isoliquiritigenin is capable of repressing lipogenesis in the liver and protecting hepatocytes from oxidative

injury inflicted by fat accumulation through a novel JNK-dependent pathway that acts as an upstream component of LXRα (unpublished data).

CYTOPROTECTIVE EFFECT OF AMPK

An energy flux is a crucial factor for cell viability. To keep the energy supply constant, eukaryotic cells use AMPK as a mechanism to monitor ATP production and expenditure. As a consequence of its sensitivity to AMP levels, AMPK is activated by treatment with drugs including metformin and TZDs as well as by conditions of metabolic stress that repress ATP production (e.g. hypoxia or glucose deprivation). Thus, AMPK activation causes upregulation of ATP-producing catabolic pathways. However, AMPK inhibits ATP-consuming pathways including synthesis of fatty acids, cholesterol, glycogen, and proteins[59]. Although AMPK signaling is intricately tied to energy metabolism and homeostasis, it is also critical for various physiological processes including inflammation, and proliferation[60,61]. It is noteworthy that the AMPK-associated pathway may suppress apoptosis induced by glucocorticoids[62], hyperglycemia[63], hepatic ischemia-reperfusion[64] and oxidative stress[65-69]. AMPK activation has a beneficial effect on cell viability *via* protection of mitochondria from apoptosis: phosphorylation of glycogen synthase kinase 3β (GSK3β)[66], and phosphorylation of Bad, which leads to inhibition of cytochrome *c* release and attenuation of caspase-3 activity[70]. AMPK is also implicated in other pathophysiological responses in various cell types: a decrease in endoplasmic reticulum (ER) stress[71], DNA damage repair[72,73], autophagy[74,75], and the antioxidant defense system[65-69]. This review focuses on the role of AMPK in hepatocyte viability.

Regulation of autophagy and cell survival

Regulation of cellular balance between biosynthesis and turnover is crucial for the maintenance of metabolic homeostasis. Autophagy is an evolutionally conserved pathway for self-digesting of cytoplasmic components and organelles by lysosomal degradation[76]. Autophagy contributes to cell survival *via* removal of long-lived proteins and damaged organelles, thus this event plays a role in adaptive protection upon starved conditions[77]. In addition, a recent study showed that autophagy regulates lipid metabolism by inducing lipid utilization in hepatocytes, implicating a possible link with metabolic diseases[78]. The autophagic processes are regulated by several signal transduction mechanisms. Among them, AMPK activation induces autophagy in response to diverse stress conditions including energy depletion, ER stress, and hypoxia. The action of AMPK is mediated by the inhibition of mTOR-dependent signaling, which is a central inhibitory pathway of autophagy[79]. The AMPK-induced autophagy exerts a cytoprotective effect, which can be regulated by upstream kinases such as LKB1[74,75]. However, the role of S6K1 inhibition by AMPK in the modulation of autophagy is unclear. Despite these primarily defensive effects, autophagy mediates cell death under certain conditions[77], thus further study would help understand the role of AMPK in autophagy-associated cell viability.

Protection of mitochondria from external stress

Insulin resistance has been associated with a reduction in mitochondrial oxidative phosphorylation and ATP production, and thus downregulates the expression of genes encoding for oxidative metabolism[80-82]. Thus, mitochondrial dysfunction is frequently observed in the metabolic syndrome[82]. Under mitochondrial dysfunction caused by several endogenous or exogenous stimulants, it is difficult to maintain redox-homeostasis. In this situation, changes in mitochondrial membrane permeability (MMP) cause the release of proapoptotic mediators that can damage DNA and lead to apoptosis[83,84]. Oxidative stress inhibits endoplasmic reticulum calcium pumps, releasing calcium into the cytoplasm from endoplasmic reticulum. The cytoplasmic calcium is taken up by mitochondria, which makes the mitochondrial permeability transition pore (mPTP)[85,86]. In basal conditions, the mPTP is closed but opens in response to stress, allowing passage of small molecules. Opening of the mPTP causes MMP transition and cytochrome *c* release, inducing apoptosis. A number of studies have shown that chemical inhibitors of the mPTP have the ability to prevent the release of cytochrome *c* and protect cells from death[87]. Excess reactive oxygen species may enhance the opening of the mPTP, and cause mitochondrial depolarization and cytochrome *c* leakage[88,89]; the release of cytochrome *c* from mitochondria to cytoplasm activates procaspase-9 and Apaf-1, and stimulates apoptosome formation and caspase-3 activation so that it induces cell death[90].

AMPK-associated signaling mediates hepatocyte viability

AMPK: AMPK responds to external stress as a modulator of cell viability or death. In many cases, AMPK activation exerts a cytoprotective effect[62-64,66]. Chemical activation of AMPK protected cells from arachidonic acid-induced apoptosis and restored MMP. In this model, cell viability depended on mitochondrial function; treatment of the AMPK activator (e.g. oltipraz and resveratrol) protected cells from mitochondrial injury. Thus, the direct or indirect AMPK activators have the ability to protect cells from mitochondrial oxidative stress. This mitochondrial protective effect could be reversed by either compound C treatment or overexpression of the dominant negative mutant of AMPKα. In our laboratory, the AMPK-dependent antioxidant and cytoprotective effects had been tested with AICAR. Cellular H_2O_2 production increased by arachidonic acid treatment impairs mitochondrial function, and promotes apoptosis. Thus, arachidonic acid propagates apoptotic signals due to oxidative stress alone or in combination with an increase in mitochondrial Ca^{2+} uptake[91]. In this model, AICAR exhibited a cytoprotective effect against injury caused by arachidonic acid so that it abolished reactive oxygen species production in the cell. The data showing that

compound C treatment induced MMP transition indicate that AMPK is necessary for MMP regulation. AMPK increases its activity through TSC2 phosphorylation, which leads to translational suppression and cell size reduction under the situation of energy deprivation. Moreover, the phosphorylation of TSC2 protects cells from apoptosis induced by energy deprivation[16], suggesting that the downstream components of AMPK may be responsible for MMP regulation.

In a recent study, resveratrol, a polyphenolic component found in grapes and red wine, was shown to protect mitochondria from oxidative stress in an AMPK-dependent manner. AMPK activation by resveratrol depended on LKB1, but not CaMKK. Thus, LKB1 activation protects cells from apoptosis under the condition of energy stress[92]. The importance of LKB1 for AMPK-dependent cytoprotection is also supported by the result of the sauchinone study: sauchinone exerted a protective effect against MMP transition *via* LKB1 activation[69]. The upstream components that activate LKB1 include SIRT1[93], nitric oxide synthase[94], and protein kinase A[12]. In addition, we identified the formation of poly (ADP-ribose) (PAR) as the upstream event, by which resveratrol activates LKB1[66]. PAR polymerase (PARP) represents a nuclear enzyme that plays a role in DNA damage repair through PAR formation. In an energetic process, PAR causes rapid depletion of NAD^+, decreases ATP production, and thus leads to cell death[75]. In contrast, PARP prevents cell death through LKB1-AMPK-mediated autophagy activation[75], which may be associated with LKB1. Sometimes, AMPK activation may cause apoptosis; sustained AMPK activation (>10 h) triggered hepatocyte death through JNK and caspase-3 activation. In this process, p53, Bax and Fas ligand are upregulated or activated by activated JNK[95]. Hence, AMPK-dependent cell survival may rely on cell type, environmental conditions and on the duration of this kinase activation[95].

Mn-superoxide dismutase (Mn-SOD) as a mitochondrial enzyme converts the superoxide anion to hydrogen peroxide, and plays a role in cytoprotection[96]. Pro-oxidants like paraquat and dinitrophenol induce Mn-SOD in the liver[97,98]. Treatment with metformin or AICAR, an AMPK activator, increases the expression of MnSOD mRNA, suggesting that Mn-SOD induction may be coupled to the AMPK-associated pathway.

GSK3β: GSK3β is a constitutively activated serine/threonine kinase in normal state. This enzyme is well known as a regulator of glycogen metabolism, gene expression, and cell cycle progression[99]. GSK3β is inactivated by serine 9 phosphorylation[100], enabling cells to suppress mPTP opening[101] and prevent apoptosis of hepatocytes[66]. Hence, this kinase may contribute to cell viability against external stress (e.g. ischemia/reperfusion injury). It has also been recognized that inhibitory phosphorylation of GSK3β prevents phosphorylation of voltage-activated anion channel, and promotes binding of GSK3β with adenine nucleotide translocase. In our study, GSK3β inhibition protected mitochondria from mPTP opening and contributed to cell survival against severe oxidative stress[66], as also supported by other reports[102,103]. This contention is supported by the finding that treatment by a direct AMPK activator (i.e. AICAR) leads to GSK3β inhibition (Figure 2), as mediated with Raf1/ERK/p90 kDa ribosomal S6 kinase[104]. Some other cytoprotective compounds also act as AMPK activators, which include resveratrol and isoliquiritigenin, and cause GSK3β inhibition[66]. Thus, GSK3β phosphorylation may lie downstream of AMPK.

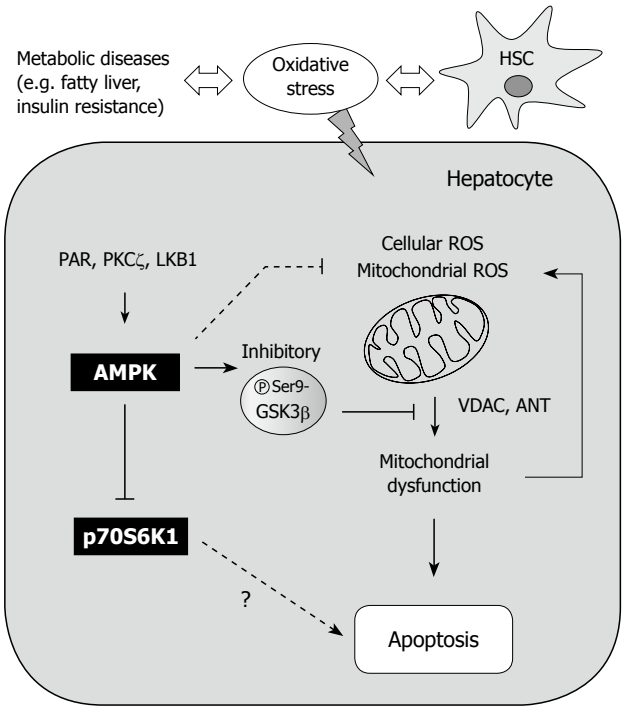

Figure 2 Adenosine monophosphate-activated protein kinase regulation of cell viability. Adenosine monophosphate-activated protein kinase (AMPK) protects cells from oxidative stress-induced H_2O_2 production and mitochondrial dysfunction, which results in part from the inhibitory phosphorylation of glycogen synthase kinase 3β (GSK3β). GSK3β inhibits mitochondrial function through voltage-activated anion channel (VDAC) phosphorylation. AMPK activation contributed to cell survival, whereas the regulatory role of S6 kinase-1 (S6K1) in apoptosis is still unclear. HSC: Hepatic stellate cell; PAR: Proliferator-activated receptor; PKC: Protein kinase C; LKB1: Liver kinase B1; ROS: Reactive oxygen species; ANT: Adenine nucleotide translocator.

PKC: In certain situations, necrosis may also be programmed through specific pathways. Hepatocytes undergo necrosis several hours after H_2O_2 treatment in association with PKC activation and/or AMPK inhibition, as evidenced by a decrease in cell death by PKC inhibitor treatment. Interestingly, PKC inhibition results in AMPK upregulation, suggesting that these two pathways are inversely coupled to each other[105]. Apparently, these pathways are linked to a cytoprotective effect, as shown by decreased H_2O_2-induced necrosis after treatment with PKC inhibitor or AMPK activator. Consistently, compound C treatment (an AMPK inhibitor) abrogated the ability of PKC inhibitor to protect cells, suggesting that PKC inhibitors have a cytoprotective effect through AMPK upregulation.

Table 1 Effects of candidate compounds on the adenosine monophosphate-activated protein kinase-S6 kinase-1 pathway and liver function

Chemicals	AMPK	S6K1	NAFLD	Hepatic insulin resistance	Cyto-protection in the liver	Effective conc.	Ref.
A class of synthetic dithiolethiones							
Oltipraz	↑	↓	+	+	+	30 μmol/L, 30 mg/kg	[27,50,67,107]
CJ11764	↑	↓	+	+	+	30 μmol/L	[27,50,67]
CJ12064	↑	↓	+	+	+	30 μmol/L	[27,50,67,107]
CJ11842	↑	↓	+	+	+	30 μmol/L	[27,50,67,107]
CJ11840	↑	↓	+	+	+	30 μmol/L	[27,50,67]
CJ11792	↑	↓	+	+	+	30 μmol/L	[27,50,67,107]
CJ11788	↑	↓	+	+	+	30 μmol/L	[27,50,67,107]
CJ11766	↑	↓	ND	+	+	30 μmol/L	[67,107]
CJ12073	↑	-	+	+	+	30 μmol/L	[27,67,107]
CJ11780	↑	ND	+	ND	ND	30 μmol/L	[27]
Metabolites of oltipraz							
M1	↑	↓	ND	+	+	30 μmol/L	[50,68]
M2	↑	ND	ND	ND	+	30 μmol/L	[68]
Phytochemicals							
Resveratrol	↑	ND	+	+	+	30 μmol/L	[66,108,109]
Isoliquiritigenin (*Glycyrrhizae radix*)	↑	-	+	ND	+	20 μmol/L, 30 mg/kg	[65], UD
Liquiritigenin (*Glycyrrhizae radix*)	↑	-	+	ND	+	100 μmol/L, 30 mg/kg	[65], UD
Sauchinone (*Saururus chinensis*)	↑	-	+	ND	+	30 μmol/L, 30 mg/kg	[69,110]
Baicalin (*Scutellaria baicalensis*)	↑	ND	+	ND	ND	10 μmol/L, 80 mg/kg	[111]

ND: Not done; UD: Unpublished data; ↑: Activation; -: No change; ↓: Inhibition; +: Beneficial effect against nonalcoholic fatty liver disease (NAFLD) or insulin resistance, or cytoprotection in the liver; AMPK: Adenosine monophosphate-activated protein kinase; S6K1: S6 kinase-1.

S6K1: In S6K1-/- hepatocytes, caspase-8 and Bid (a pro-apoptotic protein) were both down-regulated relative to control. A deficiency of S6K1 was not sensitive to the cascades of death receptor activation, as shown by no caspase-8 activation or FLIP$_L$ degradation in hepatocytes challenged by TNF-α or anti-Fas antibody treatment. The finding that Bid cleavage, cytochrome *c* release, caspase-3 activation, and DNA laddering were all attenuated by a deficiency of S6K1 raises the importance of S6K1 in the apoptotic process. Consistently, the lack of S6K1 did not diminish the BclxL/Bim ratio in cells deprived of serum, and thus prevented cytochrome *c* release and DNA fragmentation[106]. In an animal model, S6K1 deficiency enabled hepatocytes to survive against concanavalin A-induced apoptosis[106]. Inhibition of S6K1 may activate survival pathways through PI3K/Akt and ERK pathways. However, hepatocytes deficient in S6K1 underwent apoptosis on serum withdrawal when combined with PI3K or ERK inhibitor treatment[106]. In this sense, S6K1 inhibition along with Akt and ERK inhibitors, would enhance the efficacy of cancer chemotherapy for hepatocarcinoma[106]. In our oxidative stress model, rapamycin, an inhibitor of mTOR-S6K1 activity that causes dissociation of raptor from mTOR by binding FK506 binding protein 12, had no effect on apoptosis elicited by arachidonic acid + iron, suggesting that the inhibition of S6K1 alone may not be sufficient to promote cell viability. Overall, the inhibition of S6K1 may contribute to protecting hepatocytes from liver failure, and if so, it might result from improvement in insulin signaling.

AMPK REGULATION OF ENERGY METABOLISM AND CELL SURVIVAL

A series of beneficial compounds with the abilities of AMPK activation are listed in Table 1 and Figure 3, which may have liver-protective effects against external stimuli. Thus, the compounds that have modulating activities on metabolism may also have cytoprotective effects (Figure 4). In these actions, LKB1-dependent AMPK activation may be one of the key molecular pathways for cell survival. A number of studies have shown how AMPK responds to an increase in AMP as an energy sensing enzyme. In this way, it integrates diverse signal inputs, controls a number of metabolic enzymes in various cell types, and adapts cellular processes to the energy status. Since AMPK activation may not always be on the side of cell survival, the specific AMPK pathways responsible for cell viability still remain to be elucidated.

CLINICAL IMPLICATIONS

Metformin is a major drug used in the treatment of type 2 diabetes. AMPK activation by metformin suppresses hepatic glucose production and lowers blood glucose levels[47,112]. In addition, metformin has been shown to reverse fatty liver disease in humans[113,114]. TZDs belong to another important class of antidiabetic drugs that augment systemic insulin sensitivity. In diabetic patients, pioglitazone decreases hepatic fat content and increases splanchnic glucose uptake presumably through AMPK[115]. In addition, these medications may prevent simple hepatic steatosis from progressing to steatohepatitis. Although the molecular mechanism of AMPK activation by TZDs is unclear, AMPK activation is attributed to their ability to increase plasma adiponectin levels[53].

Hepatic ischemia-reperfusion injury, usually in association with liver transplantation and hepatic resection, is an important clinical issue. Ischemic preconditioning may

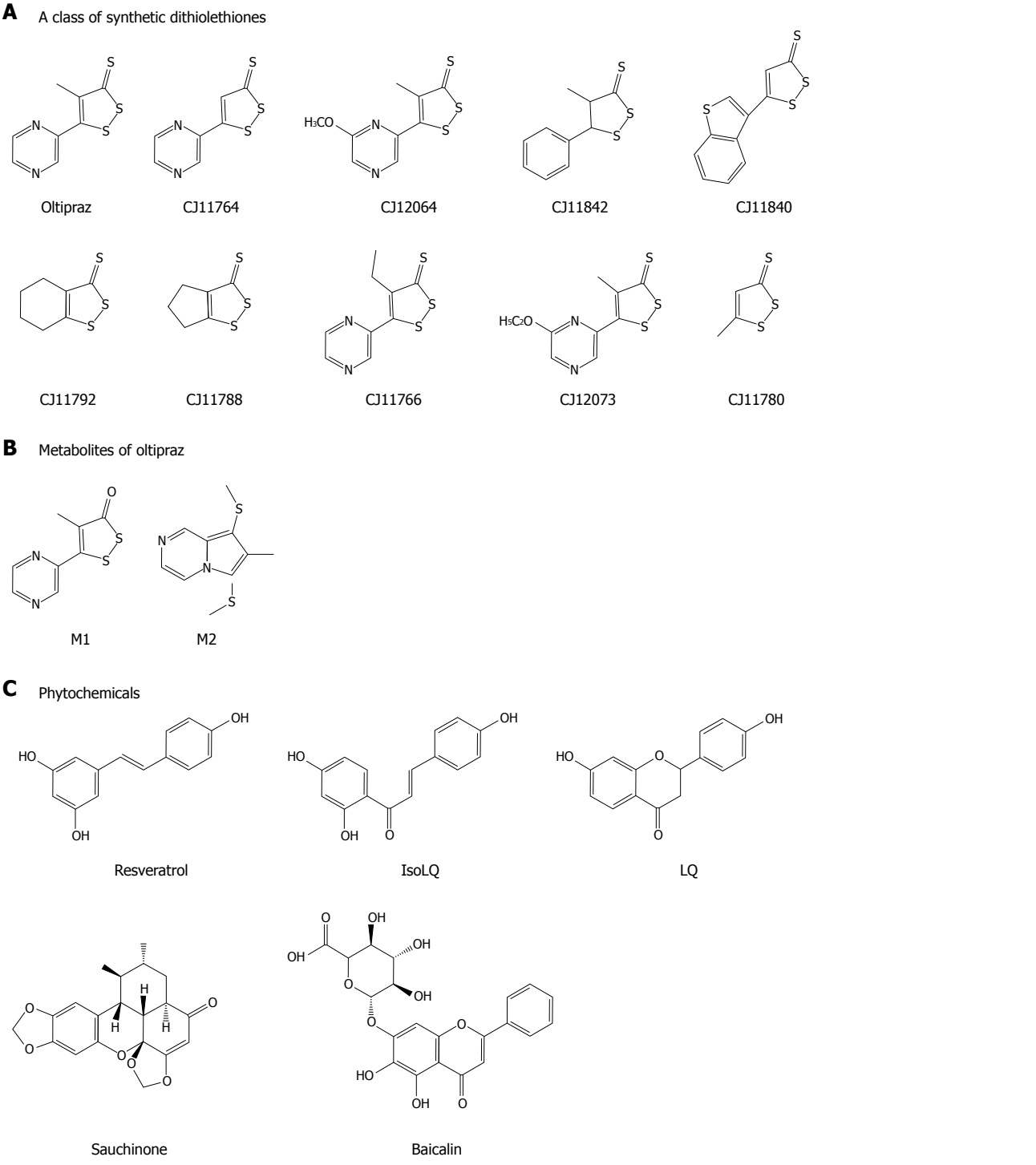

Figure 3 Chemical structures of adenosine monophosphate-activated protein kinase activators. A: Dithiolethione derivatives: Oltipraz [4-methyl-5-(2-pyrazinyl)-1,2-dithiol-3-thione], CJ11764 (5-pyrazinyl-1,2-dithiole-3-thione), CJ12064 [5-(6-methoxypyrzinyl)-4-methyl-1,2-dithiole-3-thione], CJ11842 (4-methyl-5-phenyl-1,2-dithiole-3-thione), CJ11840 (5-benzo[b]thiophene-3-yl-1,2-dithiole-3-thione), CJ11792 (4,5,6,7-tetrahydrobenzo-1,2-dithiole-3-thione), CJ11788 (5,6-dihydro-4H-cyclopenta-1,2-dithiole-3-thione), CJ11766 (4-ethyl-5-pyrazin-2-yl-1,2-dithiole-3-thione), CJ12073 [5-(6-Ethoxypyrazin-2-yl)-4-methyl-1,2-dithiole-3-thione], CJ11780 (5-methyl-1,2-dithiole-3-thione); B: Metabolites of oltipraz: First, oxidative desulfuration of the thione among approximately 1% of oltipraz to yield M1 [4-methyl-5-(2-pyrazinyl)-1,2-dithiol-3-one], which is not metabolized further; and secondly, desulfuration, methylation, and molecular rearrangement among a large amount of oltipraz to yield M2 [7-methyl-6,8-bis(methylthio)H-pyrrolo(1,2-a)-pyrazine], which is metabolized to other oxidized forms; C: Phytochemicals: Resveratrol (flavonoid found in the skin of red grapes and red wine), isoLQ (a flavonoid aglycone of isoliquiritin from licorice), LQ (a flavonoid aglycone of liquiritin from licorice), sauchinone (a lignan in *Saururus chinensis*), baicalin (the major flavonoid compound in *Scutellaria baicalensis*).

be beneficial to patients with hepatic resections in which long periods of ischemia are necessary. Ischemic preconditioning prevents ATP degradation and intracellular accumulation of AMP induced by subsequent ischemia[116]. Increases in AMP levels during ischemia activate AMPK, while AMPK inhibition abolishes the effect of preconditioning, indicating that AMPK plays a role in this effect[64]. So, hepatic preconditioning may allow the liver to

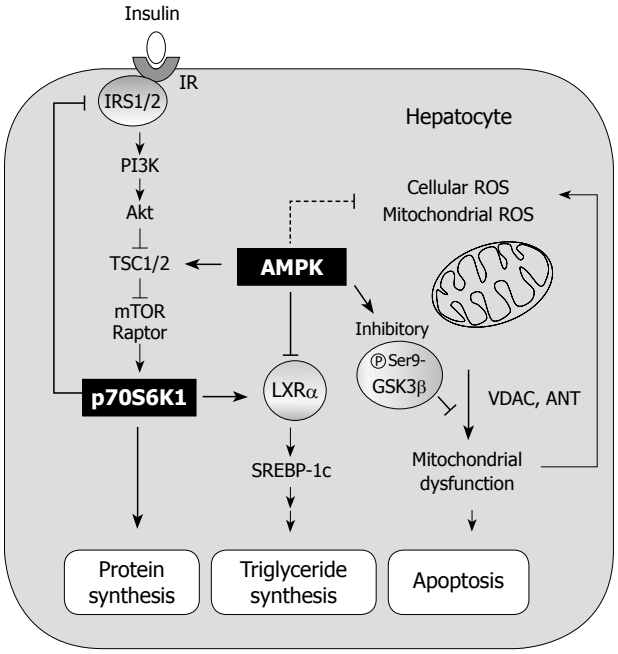

Figure 4 Dual regulation of fuel metabolism and cell viability by adenosine monophosphate-activated protein kinase. The adenosine monophosphate-activated protein kinase (AMPK)-S6 kinase-1 (S6K1) pathway regulates metabolic signaling flow. In addition, AMPK controls the redox-state and mitochondrial function, therefore its activation protects cells from apoptosis. AMPK is the key molecule to bridge the gap between fuel metabolism and hepatocyte viability. IR: Insulin receptor; IRS1: Insulin receptor substrate-1; PI3K: Phosphoinositide-3 kinase; TSC1: Tuberous sclerosis complex 1; ROS: Reactive oxygen species; mTOR: Mammalian target of rapamycin; LXRα: Liver X receptor-α; GSK3β: Glycogen synthase kinase 3β; SREBP-1c: Sterol regulatory element, binding protein-1c; VDAC: Voltage-activated anion channel; ANT: Adenine nucleotide translocator.

preserve energy metabolism during sustained ischemia[116]. Since AMPK activation by preconditioning may represent a new strategy to reduce the ischemia-reperfusion injury, modified preservation solutions containing AMPK activators may be of use, which should be evaluated in clinical settings.

CONCLUSION

As the mitochondrion plays a diverse role in essential cellular functions including energy production and homeostasis, redox cell signaling, and apoptosis, the chemical activators of AMPK protect hepatic mitochondria against toxic stress. The inhibition of S6K1 downstream of AMPK may also have a distinct role in liver biology. Thus, the AMPK pathway is associated with various pathological conditions, including metabolic syndrome and numerous apoptotic conditions. Because of the shared regulatory functions of AMPK in metabolism and cell viability, it becomes an advantageous target. In this review, we have proposed the concept that AMPK-associated signaling bridges the gap between fuel metabolism and hepatocyte viability, which may be of help in identifying valuable potential targets to prevent and/or treat derangement of metabolism and cell death in the liver.

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EDITORIAL

Gastroesophageal reflux disease: From heartburn to cancer

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Abstract

About 10%-15% of patients with gastroesophageal reflux disease develop Barrett's esophagus. This is considered a premalignant condition because it can progress from metaplasia to high-grade dysplasia, and eventually to adenocarcinoma. Recently, major advances have been made in the endoscopic treatment of Barrett's esophagus, therefore limiting the role of surgery in the treatment of this disease.

Key words: Gastroesophageal reflux disease; Barrett's esophagus; Esophageal adenocarcinoma; Laparoscopic fundoplication; Radiofrequency ablation; Esophageal endoscopic mucosal resection; Minimally invasive esophagectomy

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Gastroesophageal reflux disease affects an estimated 20% of the population in the United States. About 10%-15% of patients with gastroesophageal reflux disease develop Barrett's esophagus, which eventually can progress to adenocarcinoma, which is currently the fastest growing cancer in the United States. It is recognized that adenocarcinoma is in most cases the end stage of a sequence of events whereby the squamous esophageal epithelium is initially replaced by columnar epithelium without dysplasia. Subsequently, the metaplastic epithelium can progress to low- and high-grade dysplasia and eventually cancer[1-3].

This symposium addresses some key questions in the treatment of this disease process. The pathophysiology and diagnosis of the disease are reviewed, particularly in morbidly obese patients[4-10]. Based on the pathophysiology, the treatment of metaplasia is discussed. Special attention has been placed on new treatment modalities such as radiofrequency ablation and endoscopic mucosal resection, which have revolutionized the treatment of high-grade dysplasia and intramucosal carcinoma[11-16]. The remaining indications for esophagectomy in these cases are discussed[17]. Finally, we have reviewed what to do when invasive cancer is present, discussing the role of neoadjuvant therapy[18-20], the type of esophageal resection (transhiatal versus trans-thoracic)[21,22], and the current data available about minimally invasive esophagectomy[23,24]. The authors are both experts dedicated to the treatment of patients with esophageal disorders and have published extensively on these topics.

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TOPIC HIGHLIGHT

Marco G Patti, MD, Professor, Director, *Series Editor*

Gastroesophageal reflux disease: From pathophysiology to treatment

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Abstract

This review focuses on the pathophysiology of gastroesophageal reflux disease (GERD) and its implications for treatment. The role of the natural anti-reflux mechanism (lower esophageal sphincter, esophageal peristalsis, diaphragm, and trans-diaphragmatic pressure gradient), mucosal damage, type of refluxate, presence and size of hiatal hernia, *Helicobacter pylori* infection, and Barrett's esophagus are reviewed. The conclusions drawn from this review are: (1) the pathophysiology of GERD is multifactorial; (2) because of the pathophysiology of the disease, surgical therapy for GERD is the most appropriate treatment; and (3) the genesis of esophageal adenocarcinoma is associated with GERD.

Key words: Gastroesophageal reflux disease; Pathophysiology; Acid reflux; Non-acid reflux; Esophageal manometry; Ambulatory pH; Barrett's esophagus; Esophageal adenocarcinoma

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INTRODUCTION

Gastroesophageal reflux disease (GERD) is a very prevalent disease. Population studies have repeatedly shown GERD-related symptoms in a significant proportion of adults. The Montreal consensus conference defined GERD as "a condition which develops when the reflux of gastric contents causes troublesome symptoms and/or complications"[1]. However, this definition did not include details of the pathophysiology of the disease and its implications for treatment. The Brazilian consensus conference considered GERD to be "a chronic disorder related to the retrograde flow of gastro-duodenal contents into the esophagus and/or adjacent organs, resulting in a spectrum of symptoms, with or without tissue damage"[2]. This definition recognizes the chronic character of the disease, and acknowledges that the refluxate can be gastric and duodenal in origin, with important implications for the treatment of this disease.

This review focuses on the pathophysiology of GERD and its implications for treatment.

GERD - ROLE OF NATURAL ANTI-REFLUX MECHANISMS

Although all normal individuals experience some sort of "physiological" gastroesophageal reflux, a highly efficient barrier exists between the stomach and the esophagus. From the esophageal side, esophageal clearance is pro-

moted by peristalsis and salivary production. A valve mechanism exists between the esophagus and the stomach, formed by the lower esophageal sphincter (LES), the diaphragm, the His angle, the Gubaroff valve and the phrenoesophageal membrane.

Peristalsis

Esophageal peristalsis is an important component of the antireflux mechanism because it is the main determinant of esophageal clearance of the refluxate. Defective peristalsis is associated with severe GERD, both in terms of symptoms and of mucosal damage[3]. As matter of fact, the composite reflux score (DeMeester score)[4] includes in its calculation two indirect measurements of esophageal clearance (number of reflux episodes longer than 5 min and length of the longest episode). In addition, the average esophageal clearance time can be calculated by dividing the total minutes the pH is below 4 by the number of reflux episodes. This association also explains the high prevalence and severity of GERD in systemic diseases that affects peristalsis, such as connective tissue disorders[5].

It is known that 40%-50% of patients with GERD have abnormal peristalsis[3]. This dysmotility is particularly severe in about 20% of patients because of very low amplitude of peristalsis and/or abnormal propagation of the peristaltic waves (ineffective esophageal motility)[6]. Esophageal clearance is slower than normal, therefore, the refluxate is in contact with the esophageal mucosa for a longer period of time and it is able to reach more often the upper esophagus and pharynx. Thus, these patients are prone to severe mucosal injury (including Barrett's esophagus) and frequent extra-esophageal symptoms such as cough[6,7].

It is still unclear whether esophageal dysmotility is a primary condition that leads to GERD, or it is a consequence of esophageal inflammation. Medical therapy does not ameliorate esophageal peristalsis[8,9]. However it has been shown that effective fundoplication improves the abnormal peristalsis in most patients[10].

LES

Physiologically, the LES is a 3-4-cm-long segment of tonically contracted smooth muscle at the distal end of the esophagus[11]. It is intuitive that the LES creates a high pressure zone between the esophagus and the stomach that prevents reflux. An effective LES must have an adequate total and intra-abdominal length, and an adequate resting pressure[12]. However, a normal LES pressure does not exclude GERD, because abnormal transient relaxation might occur. Periodic relaxation of the LES in normal individuals has been termed transient lower esophageal sphincter relaxation (TLESR), to distinguish it from relaxation triggered by swallowing. TLESR accounts for the physiological reflux found in normal subjects. When it becomes more frequent and prolonged, TLESR can contribute to reflux disease, and this phenomenon appears to explain the reflux seen in the 40% of patients with GERD whose resting LES pressure is normal. What determines TLESR is unknown, but postprandial gastric distention is probably involved[11,13]. It has been shown that a mechanically incompetent LES is progressively associated with worse mucosal damage[7].

At the present time, there are no medications used in clinical practice that act on the LES. Some studies are presently conducted using inhibitors of the GABA type B receptor, especially baclofen, but the effect of this medication is still not clear. These data underline that an incompetent LES represents a mechanical and permanent defect of the gastroesophageal barrier. Only fundoplication can correct the functional and mechanical profile of the LES, therefore resulting in control of any type of reflux from the stomach into the esophagus.

Diaphragm

The crus of the diaphragm provides an extrinsic component to the gastroesophageal barrier. This pinchcock action of the diaphragm is particularly important as a protection against reflux induced by sudden increases in intra-abdominal pressure[13]. This mechanism is obviously disrupted by the presence and size of a hiatal hernia.

Increase of thoraco-abdominal pressure gradient

Abnormal gastric emptying might contribute to GERD by increasing intra-gastric pressure. Patients with suspected abnormal gastric emptying should be tested with nuclear markers[14] or ultrasound[15]. If slow emptying is diagnosed, appropriate therapy should be considered. Medication such as metoclopramide and Nissen fundoplication improve gastric emptying[16].

There is also strong evidence of a possible link between obesity and GERD. Specifically, it has been shown that there is a dose-response relationship between increasing body mass index (BMI) and prevalence of GERD and its complications[17-19]. Some studies have reported that morbidly obese patients with GERD have a higher incidence of incompetent LES, transient LES relaxation and impaired esophageal motility than non-obese patients with GERD[8,20,21]. However, a detailed mathematical analysis has shown that the severity of GERD (based on the DeMeester score) is associated with BMI[22], which suggests that obesity plays an independent role in the pathophysiology GERD, mainly through increased abdominal pressure[18,23].

The association of different pulmonary diseases and GERD has recently gained renewed interest[24]. It has been shown that patients with end-stage lung disease have a high prevalence of GERD; up to 70%[25]. Although in these patients pan-esophageal motor dysfunction is frequently found[25], a more negative thoracic pressure with an increase in the gradient between intra-gastric and intra-thoracic pressure might also contribute.

GERD: ROLE OF MUCOSAL DAMAGE

Increasing severity of esophagitis is associated with increasing acid exposure[26]; however, erosive esophagitis is present in only 50% of GERD patients[7]. Some experts believe that the erosive and non-erosive forms of the

disease might actually account for different subsets of the disease; others believe that they represent two different and progressive stages of the disease.

It is still unclear if mucosal inflammation is a cause or a consequence of GERD. Evidence has shown that esophagitis is associated with esophageal body dysmotility[7]. However, it is still unclear if it is the cause or the effect of the altered peristalsis. We do know that medical therapy for GERD does not ameliorate esophageal peristalsis[8,9], whereas surgical therapy clearly results in improvement[10].

GERD: ROLE OF THE REFLUXATE

As previously mentioned, gastric and duodenal contents can reflux into the esophagus and adjacent organs. Gastric hydrochloric acid has long been recognized as harmful to the esophagus[27]. However, gastro-esophageal refluxate contains a variety of other noxious agents, including pepsin[26]. Currently, it is recognized that this component of the refluxate (commonly called bile reflux and identified by the Bilitec bile reflux monitor using bilirubin as a marker) is composed of bile salts and pancreatic enzymes[26], and is also injurious to the esophageal mucosa. It causes symptoms[28], and could be linked to the development of Barrett's esophagus[29] and esophageal adenocarcinoma[30].

Besides the constituents of the refluxate, symptom perception and mucosal damage also appear to be linked to the patterns of esophageal exposure and the volume of the refluxate. Individuals are more likely to perceive a reflux event if the refluxate has a high proximal extent and a large volume[26].

Acid suppression is the main treatment for GERD. It has evolved from topical alkaline antacids to very effective proton pump inhibitors (PPIs). Several studies have shown the efficacy of PPIs in almost neutralizing gastric acid. These medications make the refluxate less aggressive, which leads to symptom amelioration and healing of esophagitis[31]. However, they do not stop reflux or cure GERD, as different studies with intraluminal impedance technology have shown that PPI therapy alters the pH of the refluxate but does not change the occurrence and number of reflux episodes[32,33]. Currently, there is no specific medication that controls non-acid reflux. On the other hand, fundoplication blocks any type of gastric refluxate because it restores the competence of the gastroesophageal junction.

GERD: ROLE OF HIATAL HERNIA

Hiatal hernia and GERD were once considered synonyms and hiatal hernia was considered a *sine qua non* condition for GERD to occur[34,35]. Currently, it is well known that both conditions can exist independently. However, it is recognized that hiatal hernia disrupts most of the natural antireflux mechanisms, and is considered an independent factor for GERD[26]. The simple presence of an abdominal portion of the esophagus is considered an antireflux mechanism, because it is submitted to positive abdominal pressure and acts as a valve[34]. In addition, TLESR seems to occur more frequently when a hiatal hernia is present. Not surprisingly, the presence and size of a hiatal hernia are associated with a more incompetent LES (the pinchcock action of the diaphragm is absent), defective peristalsis, more severe mucosal damage, and increased acid exposure[36].

Hiatal hernia is associated with early recurrence and failure of medical therapy for GERD[34]. The reduction of a hiatal hernia with narrowing of the esophageal hiatus is a key element in fundoplication and its omission or failure is a cause of recurrence of GERD.

GERD: ROLE OF *HELICOBACTER PYLORI*

The association of GERD and *Helicobacter pylori* (*H. pylori*) is very controversial. While some argue that the infection might play a role in the prevention of GERD by altering the nature of the refluxate (gastritis leading to achlorhydria), others find no link between the infection and esophageal diseases[37,38].

Prevalence studies seem to suggest that *H. pylori* infection is inversely associated with reflux esophagitis in some populations[37]. Eradication studies also suggest that *H. pylori* infection is protective with respect to GERD[37].

If *H. pylori* protects against GERD, a logical assumption would be that it also protects against adenocarcinoma development. Furthermore, adenocarcinoma incidence is rising worldwide; however, the increasing pace is slow in underdeveloped countries, exactly where *H. pylori* incidence is higher. Indeed, the majority of epidemiological studies have found a protective association, and the results of three recently published meta-analyses have shown that *H. pylori* colonization of the stomach is associated with a nearly 50% reduction in cancer risk[39].

GERD AND BARRETT'S ESOPHAGUS

The history of Barrett's esophagus has been complicated by different opinions on the genesis of the disease[40]. Currently, it is unquestionable that Barrett's esophagus is an acquired disease caused by GERD, although risk factors and innate predisposition are still been scrutinized. Also, it is believed that most, if not all, esophageal adenocarcinoma arises in Barrett's mucosa[41].

With regard to GERD pathophysiology, Barrett's esophagus represents an end stage form of the disease. It encompasses pan-esophageal motor dysfunction that is characterized by abnormalities in esophageal peristalsis, defective LES, and bile reflux[42]. Most authors consider this form of GERD to be a surgical disease[43], based on the aforementioned points.

FROM PATHOPHYSIOLOGY TO TREATMENT

The simultaneous use of intra-esophageal impedance and pH measurement of acid and non-acid gastroesophageal

reflux has clearly shown that treatment with PPIs only changes the pH of the refluxate, without stopping reflux through a functionally or mechanically incompetent LES[44]. For instance, using this technology, Vela *et al*[44] have shown that during treatment with omeprazole, postprandial reflux still occurs but it becomes predominantly non-acid. In a study in normal subjects, Vela and colleagues also have shown that baclofen, a GABA B antagonist, is able to reduce both acid and non-acid reflux by decreasing TLESR, the primary mechanism for both acid and non-acid reflux[45]. This study signals an important shift toward treatment focused on the competence of the LES rather than the pH of the refluxate alone. This goal can also be achieved by fundoplication; an operation that can be done laparoscopically with a short hospital stay, minimal postoperative discomfort, fast recovery time and excellent results[46-49]. Long-term studies have shown that fundoplication controls symptoms in 93% of patients after 5 years and in 89% after 10 years[46]. The operation controls reflux because it improves esophageal motility, both in terms of LES competence and quality of esophageal peristalsis[10]. Control of reflux is not influenced by the pattern of reflux, and is equally effective when reflux is upright, supine or bipositional[47]. In addition, the operation is equally safe and effective in young or elderly patients[48]. Concern has been raised about the presence of postoperative dysphagia. In our experience, this occurs in about 8% of patients, irrespective of the type of fundoplication, and it resolves spontaneously in all but a few patients in a few months, without requiring re-intervention[49].

It is important to select the best treatment for the individual patient based on a review of symptoms, age, sex, esophageal function, and type of refluxate. We feel that laparoscopic fundoplication is indicated in the following circumstances: when heartburn and regurgitation are not affected by medical treatment; when it is thought that cough is induced by reflux (Mainie *et al*[50] have shown that patients with a positive symptom index resistant to PPIs with non-acid or acid reflux demonstrated by multichannel intraluminal impedance-pH monitoring can be treated successfully by laparoscopic Nissen fundoplication); poor patient compliance; cost of medical therapy if more than one pill/day of PPI is needed (most insurance companies in the United States pay for one pill/day only); and postmenopausal women with osteoporosis. It has been shown that PPIs and histamine-2 receptor antagonists can increase the risk of hip and femur fractures[51]. Therefore, medical treatment is not advisable for young and very symptomatic patients.

Finally, in a recently published meta-analysis of medical *vs* surgical management for GERD, Wileman *et al*[52] have shown that, in adults, laparoscopic fundoplication is more effective than medical management for the treatment of GERD in the short to medium term. Surgery, however, carries some risk and its application should be individualized and the decision to undergo fundoplication should be based on patient and surgeon preference.

CONCLUSION

The pathophysiology of GERD is clearly multifactorial. While medical therapy can only affect gastric acid production, fundoplication restores the function of the LES and improves esophageal peristalsis. In addition, fundoplication stops any type of refluxate because it restores the competence of the gastroesophageal junction. It seems that fundoplication alone does not cause regression of Barrett's esophagus and does not prevent the development of adenocarcinoma. It will be important to study in patients with Barrett's esophagus the long-term effect of surgery in association with new treatment modalities such as radiofrequency ablation (RFA) and endoscopic mucosal resection (EMR). The combination should be more effective than monotherapy, because RFA and EMR eliminate the metaplastic or dysplastic epithelium, while fundoplication stops reflux, which is the original cause of Barrett's esophagus.

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TOPIC HIGHLIGHT

Marco G Patti, MD, Professor, Director, *Series Editor*

Advances in diagnostic testing for gastroesophageal reflux disease

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Abstract

Gastroesophageal reflux disease (GERD) contributes substantially to morbidity and to costs in the United States health care system. The burden of this disease has resulted in attempts at improving diagnosis and characterizing patients. Numerous research and technical advances have enhanced our understanding of both the utility and limitations of a variety of diagnostic modalities. The purpose of this review is to highlight recent advances in GERD diagnostic testing and to discuss their implications for use in clinical practice. Topics addressed include esophageal pH monitoring, impedance testing, symptom association analyses, narrow-band imaging, and histopathology.

Key words: Gastroesophageal reflux disease; pH impedance; pH monitoring; Symptom association

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INTRODUCTION

Gastroesophageal reflux disease (GERD) is a common, chronic disease that affects up to 20% of the adult population in the United States[1]. It is the most frequent digestive system diagnosis in ambulatory care and at inpatient discharge[2]. GERD contributes in excess of $10 billion in annual direct health care costs, with the majority of cost attributed to proton pump inhibitors (PPIs)[2,3]. The substantial disease burden of GERD and recognition of PPI unresponsive patients has fostered numerous efforts to improve diagnostic and therapeutic monitoring modalities.

Research investigations have enhanced our understanding of both the utility and limitations of a variety of diagnostic modalities. Newer techniques for esophageal functional testing such as wireless pH capsule monitoring, duodenogastroesophageal (also referred to as alkaline or bile reflux) reflux detection, and esophageal impedance testing have been introduced over the past decade and are utilized in clinical practice. The American College of Gastroenterology, American Society for Gastrointestinal Endoscopy and American Gastroenterological Association have recently published updated reviews and guidelines on reflux management and monitoring[4-6]. This review highlights recent advances in GERD diagnostic testing and their utility in clinical practice. A literature search was conducted for English-language articles dealing with functional evaluation of the esophagus from 2008 to 2009. Databases included Medline and PubMed, with search

terms that included esophageal pH monitoring, GERD, and esophageal impedance.

ESOPHAGEAL pH MONITORING

Wireless capsule pH monitoring: Is it better than catheter systems?

A significant advance in pH recording has been the incorporation of an antimony electrode into a wireless capsule that transmits pH data to an external receiver *via* radiofrequency telemetry (433 MHz)[7,8]. Major advantages of the wireless system include patient tolerability and capability of performing extended recording periods of 2-4 d. Discomfort associated with conventional catheter electrodes can lead patients to minimize or avoid reflux-provoking stimuli such as meals and physical activity, thus decreasing the detection of abnormal acid exposure[9,10]. As a result of improved patient tolerability, the wireless pH system might provide a more accurate picture of an individual's acid exposure profile under more realistic conditions.

Several investigations have compared wireless to catheter-based pH monitoring. A recent study has evaluated simultaneous placement of the Bravo capsule and SlimLine catheter system in 55 patients referred with GERD symptoms and 53 healthy volunteers[11]. The Slimline system was removed after 24 h while the Bravo system recorded 48 h of data. The SlimLine catheter system recorded almost double the acid exposure time than the Bravo system in both patients and volunteers. A similar finding has been noted in previous studies[12,13]. There was correlation between pH values and a concordance of diagnostic yield of 82.1%. However, the authors argue that, due to a wide variation in repeated measurements and random variation, as measured by limits of agreement, the two methods are not interchangeable[11].

It is not clear from the study methods whether the increased acid detection by the SlimLine catheter system was due to a thermal calibration artifact intrinsic to the catheter pH recording system first reported in 2005[13]. This error has since been corrected. The SlimLine system also records a greater number of reflux events than does Bravo, which is related to a higher sampling frequency. This numerical difference has previously been shown to have a minimal effect on the overall acid exposure time[13,14]. Other potential explanations for the different measurements include lost data due to interrupted signal transmission by the wireless system, and movement of the pH sensor in the catheter system relative to the esophagogastric junction. The latter factor might be important given the axial shortening of the esophagus during swallowing, which could move the catheter electrode closer to or even transiently into the proximal stomach. The Bravo system was better tolerated and preferred by patients, although the investigators did report a failure rate of approximate 15% due to failure or premature detachment.

Prolonged monitoring: Is 4 d better than 1 d?

Extended pH monitoring using wireless technology might theoretically improve the detection of reflux and increase the sensitivity of testing. Several studies have demonstrated that increasing the recording period from 24 to 48 h increases the sensitivity of pH monitoring by 10%-26%[4,8]. Several studies have also consistently demonstrated higher acid exposure values on day 2 compared with day 1 with the wireless capsule. Although the differences are generally small, this might affect the interpretation in a subset of studies[11]. Most capsules are placed immediately after endoscopy, therefore, the observation raises concerns regarding the potential impact of conscious sedation on reflux detection in the time period immediately after endoscopy, when patients might be resting and avoiding typical activity.

Another advantage of a prolonged monitoring period is the ability to perform testing both on and off PPI therapy in a single study[15,16]. Controversy exists regarding whether pH monitoring is best done off or on PPI therapy, because there are advantages and disadvantages to each approach. Off-therapy testing evaluates the presence of abnormal acid exposure and maximizes symptom-reflux association owing to the greater number of symptom and reflux episodes. Off-therapy testing is used to document the presence of acid reflux in patients with non-erosive reflux disease, who are being considered for anti-reflux endoscopic or surgical therapy. Off-therapy testing is also employed for patients with a low index of suspicion for having reflux disease, such as those showing no symptomatic response to empiric trials of PPI therapy or those with atypical symptoms. In contrast, pH testing on PPI therapy can provide documentation of the effectiveness of PPI therapy.

The feasibility of pH monitoring for an extended duration was recently determined for 96 h (48 h off PPI therapy followed by 48 h on therapy) in 60 patients[16]. A single pH capsule was placed and calibrated to two separate receivers with the second receiver activated after 48 h upon initiation of PPI therapy. Reflux symptoms were also recorded. Complete 96-h data were available for 40 patients (67%) at completion of the study, with 20 patients having incomplete data transmission or early capsule detachment. A total of 14 patients had abnormal acid exposure in the first 48 h, and day 2 testing (off therapy) increased the detection of abnormal acid exposure by 10%. On PPI therapy, 39 out of 40 patients (97.5%) had complete normalization of acid exposure at day 4. In addition, three symptom association indices [symptom index (SI), symptom sensitivity index (SSI), and symptom association probability (SAP)] all decreased by day 4 on PPI therapy. Overall, the prolonged testing increased the detection of acid exposure and reflux events for symptom association measurements and allowed for evaluation of both acid exposure and symptom response to PPI therapy. Limitations of this approach included early capsule detachment in 15% and the need for two separate receivers. Updated models of the wireless pH capsule are expected to allow for prolonged 4-d recording with a single receiver.

pH sensor location: Is 5 cm the best site?

By convention, correct positioning of the catheter pH

electrodes is 5 cm above the proximal border of the lower esophageal sphincter (LES) and 6 cm above the squamocolumnar junction (SCJ) for the wireless pH capsule. These locations minimize potential noise from proximal stomach acid exposure, at the expense of decreased sensitivity. This is a particular concern for catheter- based systems in which esophageal shortening during deglutition results in relative movement of the pH sensor closer to the LES. Grigolon *et al*[17] recently have evaluated differences in subcardial pH measured at two different locations in GERD, as well as the role of hiatal hernia. Their study population consisted of 14 healthy volunteers and 11 and 10 GERD patients with and without a hiatal hernia, respectively. Wireless pH monitoring was performed using the Bravo capsule 2 cm below the SCJ, and all patients received a standardized lunch after placement of the capsule. The investigators confirmed that subcardial pH was highly acidic in the early stage after meals, but there was no difference between healthy subjects and GERD patients. The presence of a hiatal hernia did not affect the results. The findings build upon important observations made by this group regarding the role of the "acid pocket" in the pathogenesis of GERD. In clinical practice, substantial intrapatient variability and interpatient heterogeneity have limited the utility of intragastric pH monitoring.

Another study has evaluated 48-h pH recording, off PPI therapy, immediately above the SCJ compared to simultaneous results obtained at 6 cm above the SCJ in 62 patients with reflux symptoms and 55 controls[18]. GERD patients included those with erosive disease as well as non-erosive patients with typical reflux symptoms that are responsive to PPI therapy. Using a pre-defined specificity of 90%, monitoring immediately above the SCJ increased the sensitivity from 63% to 86% in all patients. The total percentage of time that pH was < 4 for the entire 48-h study was the parameter that best discriminated between GERD patients and controls. Patients with and without esophagitis had an increased sensitivity (78% to 97% and 47% to 73%, respectively) that indicated an increased discriminatory power for patients with more severe disease. These results were similar to another study in which pH measurements were obtained simultaneously 6 and 1 cm above the gastroesophageal junction (GEJ) in 40 GERD patients with and without erosive disease[19]. The investigators found improved diagnostic accuracy in patients with erosive disease but not non-erosive reflux disease (NERD). Although the results of these studies are encouraging for increasing the sensitivity of pH testing, especially in patients with more severe disease, more validation is needed before changing the conventional location of pH measurements.

pH-IMPEDANCE TESTING

Theoretical advantages

Intraluminal impedance monitoring detects changes in the resistance to electrical current across adjacent electrodes positioned in a serial manner along a catheter. Multiple electrodes positioned along the axial length of the impedance catheter determine the proximal extent of a reflux event. It is capable of differentiating antegrade from retrograde bolus transit, as well as liquid from gas reflux. A pH electrode incorporated into the recording assembly allows for simultaneous detection of acid content. Patient tolerability is similar to conventional pH monitoring as this is a catheter- based system. Likewise, recording has been limited to 24 h.

There is considerable debate on the current role of pH-impedance testing in clinical practice[20-22]. As PPI use for GERD has increased, patients presenting with typical or atypical reflux symptoms in spite of PPI therapy, and without erosive esophagitis, often pose a diagnostic and management challenge. The association of non-acid reflux events with symptoms has been demonstrated in several studies[23-26]. Impedance-pH monitoring is the most sensitive technique for the detection of reflux events. As a result of the ability to detect, localize and classify reflux events as acidic, weakly-acidic or alkaline, simultaneously, pH-impedance testing has been posited as the future standard for reflux detection and monitoring[27]. In addition, the more comprehensive reflux detection could guide more individualized therapy in patients based on their reflux profile as well as predict response to medical or surgical treatment[20,21].

Although theoretically superior to pH monitoring, the clinical utility of combined pH-impedance monitoring is still being investigated. Conventional pH testing has demonstrated high sensitivity and specificity in patients with GERD and erosive esophagitis. The chemical nature of non-acid reflux does not allow the presence of mucosal erosions to be used in the determination of sensitivity and specificity of impedance data. Therefore, studies that have examined the utility of impedance testing have relied upon symptom-reflux association methodology to support the clinical significance of non-acid reflux. As discussed below, substantial limitations for symptom-reflux association accuracy in the evaluation of acid reflux also apply to non-acid reflux. Furthermore, the reliance on symptom indices necessitates careful delineation of the specific symptom being evaluated. For instance, symptom association for regurgitation on PPI therapy is better detected by impedance testing than pH testing alone. However, the importance of non-acid reflux in generating symptoms of heartburn or chest pain is unclear. It has been demonstrated that the majority of persistent heartburn or chest pain events on PPI therapy are not related to either acid or non-acid reflux[26,28]. Extra-esophageal symptoms of globus, asthma and hoarseness might occur independent of individual reflux events and thus are inappropriate for reflux-symptom association analysis. GERD is often considered as a cause of chronic cough. Although studies have shown symptom correlation between cough and GERD, 50% of the cough episodes precede the individual reflux events, which demonstrates that cough-induced reflux occurs as often as reflux-induced cough[28].

Further difficulties in substantiating a role for pH-impedance monitoring arise from the absence of highly

effective, pharmacological therapies for non-acid reflux. Limited studies have used baclofen and baclofen analogs that inhibit transient LES relaxation. Surgical fundoplication is a more definitive means of arresting both acid and non-acid reflux, and ongoing studies are examining the use of pH-impedance results in predicting postoperative outcomes in refractory reflux patients. Additional limitations of impedance monitoring include low baseline impedance values generated by the mucosa of Barrett's esophagus and esophagitis, which make detection of liquid reflux problematic in such circumstances. Inaccuracies in the current versions of automated analysis software require careful and time consuming manual data correction[29].

Recent data

As a result of the ability to characterize acidity and determine number, duration, and location of reflux events, the majority of research using pH-impedance has focused on the challenges associated with diagnosing and treating NERD. A recent small study has evaluated 16 NERD patients with both pH-impedance and combined multiple pH monitoring in an effort to assess changes in reflux acidity and sensitivity to reflux events[30]. Compared to multiple site pH testing (at three locations), pH-impedance monitoring showed a small increase in sensitivity in detecting proximal reflux events. The authors reported that 30% of all distal acid reflux events became weakly acidic in the proximal esophagus, and a third of these events resulted in symptoms. Although the sample size was small, the results lend support to the concept of hypersensitivity in the proximal esophagus in a subset of NERD patients[31,32].

In a much larger study, Savarino *et al*[33] have evaluated the diagnostic utility of pH-impedance monitoring in 150 patients with NERD off PPI therapy. Among patients with normal distal esophageal acid exposure time, they found similar positive symptom associations for patients with acid reflux (15%) and non-acid reflux (12%). Twenty-six per cent of this group had a negative symptom association and were considered functional heartburn patients. The classification of patients with hypersensitive esophagus accorded by pH-impedance results (normal acid exposure time, positive symptom association) reduced the number of patients that would have been classified as having functional disease by 40%[33]. However, overall 87% of the 150 NERD patients had acid reflux identified as the etiology of their symptoms.

Impedance pH monitoring has also been used to compare reflux patterns between patients with erosive esophagitis and NERD[34,35]. In a small study of 26 patients, evenly split between NERD and erosive disease, pH-impedance monitoring did not reveal significant differences in mean reflux duration or the incidence of acid or non-acid reflux episodes. When stratified by type of reflux episode, patients with erosive disease did have slightly more liquid (mean 9 ± 2 *vs* 5 ± 1, $P = 0.07$) and acid (mean 9 ± 2 *vs* 4 ± 1, $P = 0.048$) reflux episodes in the supine position. Overall, pH-impedance could not discriminate between NERD and erosive esophagitis but this likely reflects the limited power of the sample size. In another study, Savarino *et al*[35] have compared a cohort of GERD patients with erosive and non-erosive disease with a control population and demonstrated increased acid exposure times, and frequencies of acid reflux events as well as proximal esophageal reflux extension, in both GERD subsets. Patients with erosive disease had a higher frequency and increased proximal migration of acid reflux events. Notably, the frequency of non-acid reflux events and their association with symptoms were similar in both erosive and non-erosive disease. Overall, the results of these studies lend further support to the argument for monitoring both acid and non-acid reflux episodes in further characterizing GERD and potentially directing management. However, the increased diagnostic yield of pH impedance over pH monitoring alone was limited and neither study has demonstrated that the increased detection results in improved patient therapeutic outcomes.

There has also been debate about whether pH-impedance monitoring should be performed on or off PPI therapy. This has recently been addressed in a small prospective study of patients with continued GERD symptoms on twice daily PPI therapy[36]. Using a randomized, crossover study design, combined 24-h pH-impedance monitoring was performed on (twice daily) and off PPI therapy for 7 d. Neither the number nor extent of reflux episodes was affected by PPI use. There were significantly more acidic reflux episodes off PPI therapy and more weakly acidic episodes on PPI therapy. However, there was lack of concordance between the SAP for both measurements, which was likely due to the small sample size of the study.

Ultimately, the benefit of using pH-impedance monitoring in routine clinical practice depends upon its ability to guide effective medical and surgical management. A prospective series of 12 patients in Switzerland evaluated using pH-impedance monitoring before and after anti-reflux surgery (mesh-augmented hiatoplasty)[37]. Although the sample size was small, the authors found that multichannel intraluminal pH-impedance monitoring significantly increased the number of reflux episodes detected before and after surgery compared to pH testing alone. There were also more patients identified as having a positive SI in the pH-impedance group. The study has found that pH-impedance monitoring provides increased data compared to pH testing alone, however, whether this information favorably affects management and long-term patient outcomes is yet to be determined. Future therapeutic trials using inhibitors of transient LES relaxation should provide valuable insights into the clinical significance of non-acid reflux.

SYMPTOM ASSOCIATION

Available methods

Three methods have been devised to use statistical calculations to correlate symptoms with acid reflux. Symptom correlation can be separately calculated for each symptom attributable to reflux, including heartburn, regurgitation

or chest pain. The application of symptom correlation to atypical reflux symptoms such as throat pain, hoarseness, cough and asthma is problematic given the lack of temporal association between such symptoms and individual reflux events. The first method developed was the SI[38], which involves dividing the number of symptoms associated with acid reflux events by the total number of symptoms, which yields a percentage. A second approach is the SSI[39], which divides the total number of reflux episodes associated with symptoms by the total number of reflux episodes. The third approach for symptom-reflux correlation is the SAP[40]. This involves constructing a contingency table with four fields: (1) positive symptom, positive reflux; (2) negative symptom, positive reflux; (3) positive symptom, negative reflux; and (4) negative symptom and negative reflux. Fisher's exact test is then applied to calculate the probability that the observed association between reflux and symptoms occurred by chance. An SAP value > 95% indicates that the probability that the observed association between reflux and the symptom occurred by chance is < 5%.

Both the SI and SSI do not take into account the total number of reflux and symptom events. Thus, in patients with very infrequent or frequent reflux episodes or symptoms, random, temporal associations between reflux and symptoms might produce an inaccurate result. Another important distinction between the methods is that the SAP determines the statistical validity of symptom-reflux associations, whereas the SI and SSI provide information on the strength of the association.

Does it work?

Past attempts to validate the utility of the symptom indices have shown conflicting results with some groups reporting correlation with PPI response[41,42], whereas others have shown high discordance rates of the indices and mediocre specificity and sensitivity[43]. As with any test used in clinical practice, reproducibility is paramount and this issue has been addressed recently in 21 patients with GERD symptoms[44]. The SI, SSI and SAP were determined in concert with 24-h pH-impedance monitoring. The SAP and SSI showed the highest reproducibility compared with the SI. The study was performed under "real world" conditions of ambulatory monitoring, which suggested that the symptom association indices, although far from ideal, can play a role in relating symptoms to reflux episodes. The limitations of symptom association and remaining cognizant of what the three methods do not measure should be considered before applying these in clinical practice. The symptom correlation tests should be viewed as complementary information that links symptoms with reflux events, which does not ensure response to either medical or surgical therapy.

OTHER MODALITIES AND ISSUES

Narrow-band imaging

Use of narrow-band imaging (NBI) to enhance the contrast between esophageal and gastric mucosa and improve visualization of the SCJ has been studied in GERD patients. NBI has been shown to increase reproducibility in grading esophagitis[45] and the ability to detect changes in the microvasculature at the SCJ[46]. More recently, a prospective study has evaluated the use of NBI to differentiate erosive esophagitis (EE) from NERD and controls[47]. A total of 107 patients underwent endoscopy with NBI. Compared to conventional endoscopy, NBI allowed for an increased detection of micro-erosions, vascularity, and mucosal islands ("pit patterns"). In terms of differentiating patients using these criteria, EE and NERD patients had a higher prevalence of micro-erosions and vascularity compared to controls. EE and NERD patients were only differentiated by an increased vascular surface in the absence of pit patterns (sensitivity 86.1%, specificity 83.3%). Although NBI with endoscopy is unlikely to serve as a standard for the diagnosis of GERD, it could serve as an adjunct in the classification of erosive and non-erosive disease.

Histopathology

The use of histological characteristics to help diagnose GERD, and specifically NERD, has garnered increased attention and has recently been reviewed[48]. Although there are limitations to many of the studies that have evaluated histology, dilation of the intracellular space (DIS) has emerged as a promising diagnostic marker of NERD[48,49]. There is also evidence that DIS can be affected by PPI treatment, potentially serving as a clinical endpoint in therapy. However, definitive histological parameters of DIS have yet to be defined for reflux disease. Histological parameters such as basal cell hyperplasia and papillae elongation have proven less sensitive or specific for GERD, but might ultimately play a role when used in combination with DIS[48,50]. Ultimately, histopathological characteristics will likely be used in concert with other modalities to diagnose and characterize GERD better.

Eosinophilic esophagitis as a confounder

Eosinophilic esophagitis (EoE) has been increasingly diagnosed in pediatric and adult populations over the past 15 years[51]. Patients can present with a variety of symptoms including dysphagia, food impaction, heartburn, and chest pain[52,53]. However, these symptoms are not specific for the diagnosis and it can be difficult to differentiate EoE from GERD. Presently, the diagnosis of EoE is defined by the combination of clinical symptoms and histological characteristics of mucosal eosinophilia (> 15 eosinophils/high-power field)[52]. Supportive features include the presence of mucosal rings, longitudinal furrows and exudates in the esophagus. Disorders such as hypereosinophilic syndrome, connective tissue disorders, GERD, drug hypersensitivity reactions or infectious esophagitis should either be excluded or deemed non-causal in the eosinophilia.

A recent retrospective case control study has evaluated clinical, endoscopic and histological characteristics that could differentiate GERD from EoE[54]. The combination of nine characteristics (age, dysphagia, food allergy,

esophageal rings, linear furrows, white plaques, no hiatal hernia, maximum eosinophil count, and eosinophil degranulation) differentiated GERD from EoE in their population[54]. However, as GERD is prevalent in approximate 20% of the United States population, it is inevitable that many patients will have coexisting disease[52,55]. Moreover, acid reflux itself might produce tissue eosinophilia or allow for allergen sensitization[56]. A significant proportion of suspected EoE patients respond both symptomatically and histologically to PPIs, which blurs the distinction between EoE and GERD even further[57,58].

CONCLUSION

As a result of complexities in phenotypic heterogeneity and pathophysiology, there is no single gold standard diagnostic modality for GERD. pH monitoring has the greatest accuracy in patients with typical heartburn and erosive esophagitis, but unfortunately, it suffers from significant limitations when applied to atypical manifestations in NERD patients. Advances in pH monitoring, most notably wireless pH capsule technology, have improved patient tolerability and allowed for prolonged recordings that allow for both detection of acid reflux and response to therapy. The sensitivity of pH monitoring might be enhanced by pH capsule positioning closer to the SCJ, but further validation is needed because of concerns for diminished diagnostic specificity. pH-impedance has clearly increased the understanding of acid and non-acid reflux pathophysiology. When combined with symptom indices, pH-impedance detection of weakly and non-acidic reflux has the potential to provide information that might guide management. Therapeutic trials that have demonstrated the predictive value of impedance data support this practice. Recent results using NBI and histopathology are of significance. Taken together, these methods lend themselves to a reductionist view of GERD, whereas patients are classified into better-defined sub-groups. This strategy could ultimately result in more effective, individualized management of GERD and improved outcomes.

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TOPIC HIGHLIGHT

Marco G Patti, MD, Professor, Director, *Series Editor*

Gastroesophageal reflux disease and severe obesity: Fundoplication or bariatric surgery?

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Abstract

Increases in the prevalence of obesity and gastroesophageal reflux disease (GERD) have paralleled one another over the past decade, which suggests the possibility of a linkage between these two processes. In both instances, surgical therapy is recognized as the most effective treatment for severe, refractory disease. Current surgical therapies for severe obesity include (in descending frequency) Roux-en-Y gastric bypass, adjustable gastric banding, sleeve gastrectomy, and biliopancreatic diversion with duodenal switch, while fundoplication remains the mainstay for the treatment of severe GERD. In several large series, however, the outcomes and durability of fundoplication in the setting of severe obesity are not as good as those in patients who are not severely obese. As such, bariatric surgery has been suggested as a potential alternative treatment for these patients. This article reviews current concepts in the putative pathophysiological mechanisms by which obesity contributes to gastroesophageal reflux and their implications with regards to surgical therapy for GERD in the setting of severe obesity.

PARALLEL TRENDS IN GASTROESOPHAGEAL REFLUX DISEASE AND SEVERE OBESITY: CAUSALITY OR COINCIDENCE

Obesity has dramatically increased over the past few decades, with the prevalence of obesity among adults in the United States, defined as body mass index (BMI) $\geqslant$ 30 kg/m^2, increased from 13% in 1960-1962[1] to 32% in 2003-2004, with 3% of men and 7% of women classified as being severely obese (BMI $\geqslant$ 40 kg/m^2) in a recent estimate[2]. BMI itself is a strong predictor of overall mortality, with a progressive excess in mortality noted above the optimum BMI of 22.5-25 kg/m^2, due mainly to metabolic and vascular disease[3]. Indeed, the prevalence of the metabolic comorbidities that contribute to atherosclerosis appears to increase significantly with increasing BMI[4,5].

In parallel with this trend in obesity is the perception that the prevalence of gastroesophageal reflux disease (GERD) has increased as well, currently affecting between 8% and 26% of the population in the western world[6-8]. These data, however, are somewhat difficult to interpret,

as these longitudinal population-based studies rely primarily upon subjective GERD symptoms rather than physiological measures of GERD. Nonetheless, there has been a significant increase in the prevalence of serious sequelae related to GERD[9-11], including Barrett's esophagus and adenocarcinoma of the distal esophagus, which strongly suggests that the severity, if not the prevalence, of GERD is in fact increasing.

Furthermore, because the prevalence of GERD is markedly higher in overweight and obese individuals as compared to those with normal BMI[12,13], GERD itself is now recognized as obesity-related comorbidity. Indeed, the importance of the relationship between excess visceral adiposity and GERD is demonstrated by the greater correlation between GERD and waist circumference and waist-to-hip ratio (markers of central obesity) than that between GERD and BMI[14]. However, the prevalence of GERD, even in the setting of severe obesity, is < 50%[15], which suggests that severe obesity itself is not sufficient to cause GERD, and that in the majority of severely obese individuals, at least some of the physiological mechanisms that prevent GERD remain reasonably intact. As such, when managing GERD in a severely obese patient and considering surgical therapy, it is useful to review the proposed mechanisms by which obesity contributes to GERD pathophysiology.

ROLE OF SEVERE OBESITY IN GERD PATHOPHYSIOLOGY

Fundamental to the development of GERD is a failure of the anti-reflux barrier that comprises the lower esophageal sphincter (LES) and the crural portion of the hiatus. LES function is directly dependent on intrinsic LES pressure (LESP, normal, 10-24 mmHg), total LES length, intra-abdominal LES length, and the frequency and duration of transient LES relaxation (TLESR). Indirectly, LES function is affected by the pressure gradient between the intragastric and intraesophageal environment.

When compared to healthy asymptomatic control subjects, 43 consecutive severely obese patients were found to have a lower LESP (11.9 ± 5.3 mmHg *vs* 15.9 ± 2.7 mmHg), and 51% were noted to have abnormal acid exposure[16]. Similarly, in a large cohort of patients with foregut symptoms, the prevalence of a mechanically defective LES (based on hypotensive LES, total length, or abdominal length) increased as BMI increased, with 55% of obese patients demonstrating a defective LES[17]. While nearly 30% of the 1659 subjects in this study were noted to be obese, specific data regarding severely obese individuals were not described. In contrast, in another large cohort of patients with GERD, mean LESP was in fact significantly greater in subjects with severe obesity (17 ± 9.2 mmHg *vs* 14 ± 7.6 mmHg), and 62% of severely obese subjects with GERD had a normal (39%) or hypertensive (23%) LES compared to only 46% of individuals with BMI ⩽ 35 kg/m^2; 10% of whom were noted to have a hypertensive LES[18]. The authors of this study hypothesized that the mechanisms responsible for GERD might be different in the setting of severe obesity, and that the observed increased LESP could represent a compensatory mechanism against the increased pressure gradient between the stomach and esophagus, which ultimately remains inadequate to prevent GERD. This finding also has important implications with regards to surgical therapy, as conventional anti-reflux procedures (i.e. fundoplication) seek to correct the defective LES.

TLESR could be the most important reflux mechanism in the setting of a functioning LES, and it has been observed that fundoplication reduces the frequency of TLESR[19]. Based on high-resolution manometry and concurrent fluoroscopy in non-obese patients, the key events that lead to opening of the gastroesophageal junction during TLESR include LES relaxation, crural diaphragm inhibition, esophageal shortening, and a positive pressure gradient between the stomach and the gastroesophageal junction lumen[20]. Obese individuals without GERD were noted to have an increased frequency of TLESR (7.3 ± 2.0 events/2 h *vs* 2.1 ± 1.2 events/2 h) compared to normal weight individuals, whereas LESP and LES length were similar between the two groups[21]. Similar findings have been noted in the setting of severe obesity[22].

Several factors might contribute to the increased gastroesophageal gradient seen with obesity[23], including increased intra-abdominal pressure[24], increased intragastric pressure[25], increased negative inspiratory intrathoracic pressure[26], and a mechanical separation between the LES and the extrinsic compression provided by the diaphragmatic crura[23]. The latter is a key step in the development of hiatal hernia, which, based on endoscopic evidence, is more prevalent in obese individuals than normal weight individuals[27,28]. Indeed, the negative effects of the presence of hiatal hernia on LES function might in fact be greater than the effects of obesity *per se*[17].

SURGICAL TREATMENT OF GERD

There is substantial controversy regarding the long-term efficacy and durability of fundoplication in the setting of obesity, and fewer data still to inform clinicians as to its effectiveness in the setting of severe obesity. A major concern regarding the long-term durability of fundoplication in severe obesity is the presumed increased risk of hiatal hernia recurrence, projected from the well-recognized contribution of obesity to the risk of hernia recurrence following abdominal wall hernia repair[29,30]. In a study of 224 consecutive patients with 3 years follow-up who underwent laparoscopic Nissen or transthoracic Belsey Mark Ⅳ (BM-Ⅳ) fundoplication, overall symptomatic recurrence was 31.3% in obese patients (22.9% Nissen, 53.8% BM-Ⅳ), compared to 4.5% in normal-weight individuals[31]. In another cohort study, preoperative severe obesity was associated with a higher rate of fundoplication failure, defined as the need for reoperation, lack of satisfaction, or severe symptoms at follow-up[32]. This study was limited by the small number of severely obese patients (only seven out of 166) and loss of patients to follow-up.

In another study of patients who were undergoing gastric bypass after failed fundoplication, the majority of failures were found to be due to wrap disruption rather than intrathoracic wrap migration[33]; the latter being the most common anatomical failure in normal and overweight patients. In contrast, several studies have demonstrated short-term and medium-term outcomes in obese patients that are comparable to those in non-obese patients[34-37]. These data are somewhat limited in their applicability to severely obese individuals, however, due to their lack of physiological outcomes measures, small numbers of severely obese patients, and relatively short follow-up period.

BARIATRIC SURGERY AND GERD

Bariatric surgery has become a widely accepted form of treatment for severe obesity, and several studies have demonstrated a significant reduction in GERD symptoms and medication utilization, as well as weight and metabolic comorbidity, including diabetes, hypertension and dyslipidemia[38,39]. Indeed, given the frequent presence of these and other comorbidities in the setting of severe obesity, the importance of significant and sustained weight loss for the overall health of severely obese patients, and the conflicting data regarding the outcomes of fundoplication in severe obesity, bariatric surgery is increasingly being seen as a more appropriate surgical treatment for GERD in severe obesity, even though objective measures of GERD outcomes might be comparable between fundoplication and gastric bypass[40]. Earlier concerns regarding the comparative safety of bariatric surgery (gastric bypass in particular) and Nissen fundoplication have been addressed by the recent finding that the morbidity and mortality rates of the two procedures were very comparable when using the University Health System Consortium database to identify morbidly obese patients who underwent laparoscopic gastric bypass (n = 21 156) or laparoscopic Nissen fundoplication (n = 6108) at American academic medical centers between 2004 and 2007[41]. Instead, discussion today is centered around the differential effects of currently performed bariatric operations [Roux-en-Y gastric bypass (RYGB), adjustable gastric banding (LAGB), biliopancreatic diversion with duodenal switch (DS), and sleeve gastrectomy (SG)] on GERD, as well as other obesity-related comorbidity.

RYGB AND GERD

RYGB accounts for over half of the currently performed bariatric operation in the United States, and appears to have a very favorable impact on GERD[42-45]. Its recognized effectiveness has even led to its use in non-severely obese patients with GERD[46], particularly in the setting of failed fundoplication[33]. Its efficacy in treating GERD is thought to be related to the relatively low acid production of the small-volume (15-30 mL) gastric pouch[47], reduction of esophageal biliopancreatic refluxate by use of a roux limb measuring at least 100 cm in length[48,49], and weight loss. The physiological effects of the anatomic configuration of RYGB, and specifically, the configuration of the gastric pouch, might in fact be a more important contributor to reflux improvement than reducing alkaline bile reflux or weight loss. When comparing GERD remission as measured by symptom resolution and medication discontinuation, super-obese patients (BMI $\geqslant$ 50 kg/m^2) who underwent RYGB had a higher rate of GERD resolution than those who underwent DS, despite the greater weight loss seen in the latter group[15].

LAGB AND GERD

Since its FDA approval in 2001, LAGB has rapidly become a popular bariatric surgical option for patients and surgeons due to its relative technical simplicity, perceived advantageous safety profile, and lack of gastrointestinal tract division or reconstruction (and consequent malabsorption). The effects of LAGB on GERD are conflicting, however, with some studies demonstrating improvement in physiological GERD metrics[16], while others show improvement on GERD questionnaires and/or through the discontinuation of GERD medications[50,51]. In contrast, several studies have demonstrated measured exacerbation of esophageal acid exposure, GERD symptoms, and the development or worsening of esophageal dysmotility following LAGB[52-54]. The mechanism by which LAGB may improve GERD is not well characterized, but is thought to include weight loss, increase in LESP, and reconstitution of the angle of His. It has been hypothesized that the poorer GERD outcomes following LAGB might be attributable to an unrecognized hiatal hernia at the time of initial band placement, which has led some to suggest that the presence of hiatal hernia is a contraindication to LAGB[55], whereas others have suggested that aggressive identification and concomitant repair of hiatal hernia improves outcomes and reduces the need for reoperation due to band slippage or pouch dilation[56]. Given these conflicting data, most bariatric surgeons do not recommend LAGB to severely obese patients with significant GERD, particularly in the setting of hiatal hernia.

SG, DS AND GERD

SG is a restrictive procedure initially described as the first procedure of a two-staged duodenal switch operation in very-high-risk super-obese patients, and is rapidly gaining popularity as a stand-alone bariatric operation. As with LAGB, early data regarding the impact of SG on GERD are mixed[57], and very little long-term or comparative data regarding SG and GERD are available. While the resection of a substantial portion of the parietal cell mass, significant weight loss, and a possibly increased rate of gastric emptying might all contribute to improvement in GERD physiology, the relatively long and narrow anatomical configuration of the sleeve might increase resistance to esophageal emptying of physiological amounts of reflux, and the parietal cell mass remains significantly greater than that with RYGB. Furthermore, when bile reflux is

controlled as a factor (through biliopancreatic diversion in the setting of DS), symptomatic resolution of GERD is greater with RYGB[15]. As such, SG in the setting of significant GERD should be recommended with caution.

SEVERE OBESITY AND GERD: SURGICAL RECOMMENDATIONS

When surgical treatment of GERD is indicated in a severely obese patient, bariatric surgery rather than fundoplication should be strongly considered. Not only does bariatric surgery, and RYGB in particular, better address the mechanisms that lead to GERD in obese patients with the potential for greater durability, but it also addresses concomitant obesity-related comorbidity by achieving significant and sustained weight loss. Therefore, in this case, the surgeon has the opportunity to substantially improve the patient's quality of life, positively impact multiple chronic medical conditions, and possibly reduce the excess long-term mortality risk associated with severe obesity in an acceptably safe, minimally-invasive, and cost-effective manner. For many patients, this discussion might be the first in which bariatric surgery is introduced as a possible therapeutic option, and it is not uncommon for patients to express significant resistance to the idea. In other instances, patients might have been considering bariatric surgery but were hesitant to discuss the possibility with their primary care physician and are receptive to the opportunity to learn more about the procedures. Not uncommonly, this discussion might require several office visits with the surgeon, and it is important that, in addition to offering detailed information regarding the procedures, the severely obese patient with GERD undergoes multidisciplinary evaluation as do other potential bariatric surgery patients, given the need for life-long changes in eating and behavior, and the need for long-term medical follow-up and vitamin supplementation. In doing this, the surgeon can provide a therapy that goes significantly beyond treatment of GERD.

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TOPIC HIGHLIGHT

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Pathophysiology and treatment of Barrett's esophagus

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Abstract

Gastroesophageal reflux disease (GERD) affects an estimated 20% of the population in the United States. About 10%-15% of patients with GERD develop Barrett's esophagus, which can progress to adenocarcinoma, currently the most prevalent type of esophageal cancer. The esophagus is normally lined by squamous mucosa, therefore, it is clear that for adenocarcinoma to develop, there must be a sequence of events that result in transformation of the normal squamous mucosa into columnar epithelium. This sequence begins with gastroesophageal reflux, and with continued injury metaplastic columnar epithelium develops. This article reviews the pathophysiology of Barrett's esophagus and implications for its treatment. The effect of medical and surgical therapy of Barrett's esophagus is compared.

Key words: Gastroesophageal reflux disease; Barrett's esophagus; Lower esophageal sphincter; Esophageal motility; Proton pump inhibitors; Antireflux surgery

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INTRODUCTION

Gastroesophageal reflux disease (GERD) affects an estimated 20% of the population, and with direct and indirect costs exceeding $10 billion annually, it is the costliest gastrointestinal disorder in the United States[1]. Much of this extraordinary sum goes to pay for increasingly more potent and widely prescribed medications to suppress gastric acid production. While these medications have been proven to relieve heartburn symptoms and heal esophagitis, they have not substantially altered the malignant complications of reflux disease. Adenocarcinoma of the esophagus, which occurs as a consequence of chronic gastroesophageal reflux, is increasing faster than any other cancer in the United States, and has surpassed squamous cell as the most prevalent type of esophageal cancer[2].

The esophagus is normally lined by squamous mucosa, therefore, it is clear that for adenocarcinoma to develop, there must be a sequence of events that results in transformation of the normal squamous mucosa into columnar epithelium. This sequence begins with gastroesophageal reflux, and with continued injury metaplastic columnar epithelium develops. Currently, in the Unites States, only an endoscopically visible segment of columnar mucosa that contains goblet cells on biopsy is considered to be premalignant, and patients with this condition are considered to have Barrett's esophagus. Barrett's esophagus is the precursor lesion for esophageal adenocarcinoma.

EPIDEMIOLOGY

The prevalence of Barrett's esophagus appears to be increasing in the Western world. It has been debated wheth-

er this represents a true rise in incidence or is secondary to a heightened awareness of the dangers of reflux disease among practitioners, and an increased use of upper endoscopy to evaluate patients with reflux symptoms[3]. The most convincing epidemiological evidence that the prevalence of Barrett's esophagus is actually increasing comes from a recent study in the Netherlands using their Integrated Primary Care Information database, which contains > 500 000 computerized patient records. In that study, there was a linear increase in the diagnosis of Barrett's esophagus that was even more pronounced if the increase was based on the number of upper endoscopies performed during the same time period (from 19.8/1000 upper endoscopies in 1997 to 40.4/1000 upper endoscopies in 2002)[4]. Epidemiological studies in England have also demonstrated an age-specific increase in the prevalence of Barrett's esophagus per 100 upper endoscopies during the years 1982-1996[5].

Thus, there is evidence that the prevalence of Barrett's esophagus is increasing, but it is clear that the true prevalence of Barrett's esophagus in the population is unknown, and likely much higher than would be expected based on clinical cases diagnosed by upper endoscopy. In one of the few autopsy studies that has evaluated the prevalence of Barrett's esophagus, Cameron *et al*[6] found 376 cases per 100 000 people in Olmsted County, MN, USA. This rate was five times higher than the clinical prevalence of Barrett's esophagus in this same area (82.6 per 100 000). Further support for the concern about a large sub-clinical population of individuals with Barrett's esophagus comes from a study done in veterans by Gerson *et al*[7]. They performed upper endoscopy in a group of patients who presented for routine sigmoidoscopy for colorectal cancer screening; none of whom had symptoms of reflux. Although there are obvious limitations to a study done primarily in older, white male military veterans, nonetheless, their finding that 25% of patients had Barrett's esophagus is concerning because, on the basis of symptoms, none of these patients would have been recommended to have upper endoscopy. These observations suggest that the majority of individuals with Barrett's esophagus go undiagnosed, either because they ignore minor reflux symptoms or, as the study in veterans suggests, they are truly asymptomatic.

PATHOPHYSIOLOGY

Overview

The development of Barrett's esophagus is likely a two-step process. The first step involves the transformation of normal esophageal squamous mucosa to a simple columnar epithelium called cardiac mucosa. This occurs in response to chronic injury produced by repetitive episodes of gastric juice refluxing onto the squamous mucosa. The change from squamous to cardiac mucosa likely occurs relatively quickly, within a few years, while the second step, the development of goblet cells indicative of intestinal metaplasia, proceeds slowly, probably over 5-10 years[8]. Once present, Barrett's esophagus can progress to low- and high-grade dysplasia, and ultimately to adenocarcinoma. This entire process is commonly described as the Barrett's metaplasia-dysplasia-carcinoma sequence.

Step one: Transition from squamous to columnar-lined esophagus

To understand what constitutes a columnar-lined esophagus an understanding of the anatomy and histology of the normal gastroesophageal junction is required. Unfortunately, the very definition of what is normal in this area remains controversial, with much debate centered on whether cardiac mucosa is normally present at the gastroesophageal junction. Although our understanding is gradually improving, Hayward's remark in 1961 that "the lower end of the esophagus is a region where the pathology, the physiology, and even the anatomy are not quite clear" remains appropriate even today[9]. In one of the first reports describing the normal gastroesophageal junction, Hayward indicated that a junctional or buffer zone of columnar mucosa is normally interposed between the acid-secreting oxyntic gastric mucosa and the acid-sensitive squamous esophageal mucosa[9]. Although an appealing concept, Hayward provided no data in support of his theory, and did not discuss the role of the lower esophageal sphincter which had been demonstrated to exist before his publication. According to Hayward, this junctional mucosa is normally found in a length of up to 2 cm at the gastroesophageal junction. He also noted the following about this junctional mucosa: (1) it was histologically distinct from normal gastric fundic and pyloric epithelium; (2) it did not secrete acid or pepsin but was resistant to both; (3) it was not congenital but acquired; (4) it was mobile and varied in length - creeping progressively higher into the esophagus with continued gastroesophageal reflux; and (5) it was potentially reversible with correction of reflux. Furthermore, he pointed out that it was located in the esophagus, and that it developed in association with gastroesophageal reflux[9].

Now, over 40 years later, there is still dispute about the histology of the normal gastroesophageal junction, but it is clear that normally there is none or at most 4 mm of cardiac mucosa in the distal esophagus at the gastroesophageal junction[10-13]. Longer lengths of cardiac mucosa are acquired secondary to chronic gastroesophageal reflux[14,15]. Supporting evidence for the concept that cardiac mucosa is acquired comes from both clinical and experimental studies. Experimental evidence comes from a 1970 study by Bremner *et al*[16] in which a series of dogs underwent stripping of the distal esophageal squamous mucosa, with or without creation of a cardioplasty to destroy the function of the lower esophageal sphincter. Squamous re-epithelialization occurred in those animals without gastroesophageal reflux, whereas in the animals with reflux after cardioplasty, the esophagus was re-epithelialized by a columnar epithelium that lacked parietal cells - the equivalent of cardiac mucosa in humans[16]. There is also clinical evidence in humans that columnar mucosa can replace normal esophageal squamous epithelium in the setting of gastroesophageal reflux. Following

an esophagectomy with gastric pull-up, reflux of gastric juice into the residual esophagus is common because there is no lower esophageal sphincter and a large hiatal hernia has been created. Postoperative endoscopy has revealed that many of these patients develop columnar epithelium that, on histology, is identical to cardiac mucosa proximal to the anastomosis in the residual esophagus, in what had pathologically been proven to be squamous mucosa at the time of the operation. Several series have revealed that this process is common, and occurs in ⩾ 50% of patients after esophagectomy with gastric pull-up, and that the length of columnar mucosa increases with longer follow-up[8,17-20]. Furthermore, the cardiac mucosa that develops in these patients proximal to the esophagogastric anastomosis has been shown to be biochemically similar to cardiac mucosa found in non-operated patients at the native gastroesophageal junction[17]. Additional support for the concept that cardiac mucosa is acquired comes from the fact that it is not found anywhere else in the gastrointestinal tract, and when present at the gastroesophageal junction, it is always inflamed and demonstrates reactive changes unrelated to either *Helicobacter pylori* infection or mucosal pathology elsewhere in the stomach[21]. This is atypical for a normal epithelium. Lastly, the presence of cardiac mucosa can be correlated with objective markers of GERD, including an incompetent lower esophageal sphincter, increased esophageal acid exposure on 24-h pH monitoring, a hiatal hernia, and erosive esophagitis[15].

The earliest manifestation of GERD might in fact be the presence of microscopic foci of cardiac mucosa at the gastroesophageal junction. This leads to the question of why the finding of a microscopic length of cardiac mucosa at the gastroesophageal junction is so common even in patients without the typical reflux symptoms of heartburn or regurgitation. This is likely to be related to the pathophysiology of early reflux disease. Evidence is accumulating that reflux disease begins with gastric distention after large and particularly fatty meals. Gastric distension leads to effacement of the lower esophageal sphincter and exposure of the squamous mucosa at the distal extent of the sphincter to gastric juice. The pathophysiology of the gastroesophageal junction has been best studied by Fletcher and McColl. They have noted that the gastric distension that occurs with eating can cause the lower esophageal sphincter to unfold by almost 2 cm in normal volunteers[22]. Moreover, they have identified an unbuffered acid pocket at the gastroesophageal junction following a meal; a phenomenon that they have attributed to gastric juice floating upon a lipid layer after ingestion of fatty food. By pulling back a pH catheter before and after a meal, they have been able to show that the pH step-up that corresponds to the functioning lower esophageal sphincter moved proximally with gastric distension, secondary to unfolding of the distal portion of the sphincter. By measuring acid exposure with a pH catheter positioned at the squamocolumnar junction, and another located 5.5 cm proximal to the squamocolumnar junction, Fletcher *et al*[23] have demonstrated significantly greater acid exposure at the squamocolumnar junction (median total percentage time pH < 4 of 11.7% *vs* 1.8% at 5.5 cm proximal to the squamocolumnar junction). This study has confirmed the presence of significant acid exposure at the most distal intrasphincteric segment of the esophagus in patients with otherwise normal acid exposure proximally at 5.5 cm above the squamocolumnar junction. These findings were subsequently extended when it was demonstrated that salivary nitrite is rapidly converted into nitric oxide when it comes in contact with gastric acid that contains physiological levels of ascorbic acid, and this reaction has been found to be maximal at the gastroesophageal junction[24]. The levels of nitric oxide generated at the gastroesophageal junction are potentially mutagenic, and might play a role in the pathophysiology of this region.

It is likely that continued injury to the distal esophagus and lower esophageal sphincter leads to progressive loss of the abdominal length of the sphincter. What started as transient sphincter unfolding with gastric distension gradually progresses to permanent sphincter destruction. With destruction of the sphincter, reflux disease is allowed to explode into the esophagus, and can lead to an increase in the length of cardiac mucosa, either as tongues or as a circumferential replacement of the distal esophageal squamous mucosa. This leads to progressive migration of the squamocolumnar junction proximally[25,26]. Confirmation of esophageal submucosal glands deep to areas lined by cardiac mucosa provides clear evidence that the development of cardiac mucosa is occurring in the esophagus in areas previously covered with squamous mucosa and not in the proximal stomach[26].

The precise details of the molecular mechanism by which squamous mucosa is transformed into cardiac mucosa remain unknown. However, there is likely to be a crucial interaction between normally sequestered esophageal stem cells and an intraluminal stimulus that drives this metaplastic process. Tobey *et al*[27] have demonstrated that exposure of esophageal squamous mucosa to gastric juice produces dilated intercellular spaces that allow molecules of up to 20 kDa to permeate down to the stem cells in the basal layer. Perhaps the sensation of heartburn occurs as a consequence of diffusion of hydrochloric acid through these intercellular spaces and stimulation of sensory afferent nerves[28]. These ultrastructural changes occur before gross or microscopic changes become apparent. Thus, one possibility is that factors present in the refluxed juice that gain access to the basal layer stem cells via these dilated intercellular spaces induce a phenotypic transformation such that cardiac columnar mucosal cells rather than squamous cells are produced.

Step two: Intestinalization of cardiac mucosa

Cardiac mucosa is thought to be an unstable epithelium, in part because of the severe inflammatory and reactive changes present on histology. It is hypothesized that cardiac mucosa progresses down one of two possible pathways, based on a combination of environmental and genetic factors. One pathway involves the expression of gastric genes and leads to the formation of parietal cells

within glands below the cardiac mucosa. Gastric differentiation leads to a mucosa called oxyntocardiac mucosa, and this is thought to represent a regressive or favorable change because oxyntocardiac mucosa is not premalignant, and appears to be protected from developing intestinal metaplasia. In the second pathway, expression of intestinal genes causes the formation of goblet cells within cardiac mucosa. In contrast to gastric differentiation, intestinal differentiation represents a progressive or unfavorable change because this mucosa is premalignant. Both oxyntocardiac mucosa and Barrett's esophagus have less inflammation than cardiac mucosa, which suggests that these mucosal types are more stable epithelia[29].

The development of goblet cells marks the transformation of cardiac mucosa into intestinal metaplasia. When an endoscopically visible length of this mucosa is present in the esophagus, the definition of Barrett's esophagus has been met. While gastroesophageal reflux is known to be the primary factor responsible for the development of Barrett's esophagus, the specific cellular events that lead to the transformation of cardiac mucosa into intestinalized cardiac mucosa are unknown. However, evidence is accumulating that intestinalization requires a specific condition or stimulus, and that Barrett's esophagus occurs in a stepwise process. The first step, from squamous to cardiac mucosa, is likely to occur in response to acid reflux. The second step, development of intestinal metaplasia, is likely to occur in response to a different type of luminal insult. Numerous studies have demonstrated that, although isolated acid reflux can cause esophagitis, Barrett's esophagus is associated with the presence of a mixture of acid and bile salts[30-32]. Furthermore, clinical experience dating back 30 years has suggested a role for refluxed bile in the development of intestinal metaplasia. In 1977, Hamilton and Yardley observed the development of columnar mucosa and intestinal metaplasia above the esophagogastric anastomosis in a group of patients after esophagectomy. They noted that "severe symptoms of gastroesophageal reflux and bile staining of the refluxed material were documented only in the group with Barrett's. In addition, pyloroplasty had been performed more commonly in this group."[33]. Recently, in two separate analyses of patients with reflux with and without Barrett's esophagus, we found that the factor most associated with the presence of Barrett's esophagus in both men and women with GERD was abnormal bilirubin reflux, as determined by Bilitec monitoring[34,35].

Fitzgerald *et al*[36] have reported several interesting observations on how the dynamics of mucosal exposure to luminal contents might affect columnar epithelial cell proliferation and differentiation. Using cultured human Barrett's esophagus biopsy specimens, they have demonstrated that continuous exposure to acidic media at pH 3.5 resulted in increased villin expression (a marker for epithelial cell differentiation) and reduced cell proliferation. Villin expression was not detected when the culture medium was made more acidic (pH < 2.5). In contrast, a dramatic increase in proliferation occurred when the Barrett's esophagus tissue was exposed to a short (1 h) pulse of acidic medium (pH 3.5) followed by a return to neutral pH. Clinically, this same group has noted that effective acid suppression results in a shift of the Barrett's epithelium away from proliferation and toward differentiation[37]. However, the cellular consequences of duodeno-gastroesophageal reflux in the setting of gastric alkalization with acid suppression medications were not addressed in that study.

It has been hypothesized that the mechanism by which acid and bile interact to cause Barrett's esophagus is related to the ionized state of bile salts[38]. It appears that in a weakly acidic environment certain bile acids are particularly toxic. At pH 3-6, these bile salts are soluble and non-ionized, and can enter mucosal cells, accumulate, and cause direct cellular injury[39]. When the luminal pH is higher than the pKa, these same bile acids are ionized and cannot cross the phospholipid membrane. Further, when the luminal pH is lower, as normally it is in the stomach, bile acids precipitate out of solution and are harmless[40]. Thus, it is only at this critical pH range of 3-5 that certain bile acids become non-ionized and able to cross the cell membrane. Once inside the cell, the pH is 7 and the bile acids become ionized and are trapped inside the cell where they have been shown to result in mitochondrial injury, cellular toxicity and mutagenesis[41-44]. Consequently, this mid-range gastric pH of 3-5 is a danger zone for patients with duodeno-gastroesophageal reflux.

It remains uncertain whether the transformation of cardiac mucosa to intestinalized cardiac mucosa represents a phenotypic change secondary to the induction of genes, or a mutational event within the columnar cells. Mendes de Almeida and colleagues have demonstrated biochemically that both cardiac mucosa and intestinal metaplasia express sucrase-isomaltase and crypt cell antigen - two small intestine marker proteins; however, in that study only three patients with cardiac mucosa were evaluated[45]. Das has developed a murine monoclonal antibody (DAS-1) that reacts specifically with normal colonic epithelial cells, and subsequently he has found that it also reacts with an unknown epitope in Barrett's mucosa[46]. Griffel *et al*[47] have reported that the DAS-1 antibody stained cardiac mucosa without intestinal metaplasia in seven patients, and that six of these patients later developed histological evidence of intestinalization on repeat biopsies. Likewise, we noted that the pattern of immunostaining with cytokeratins 7 and 20 was similar in cardiac mucosa and Barrett's esophagus[48]. These findings suggest that, biochemically, cardiac mucosa and intestinal metaplasia are similar, and that cardiac mucosa is the precursor of intestinalized columnar epithelium, or Barrett's esophagus.

Currently, the length of Barrett's esophagus is divided into short (< 3 cm) and long (≥ 3 cm) segments based on the endoscopically determined length of the columnar streak or column in the distal esophagus. Clinically, patients with long-segment Barrett's esophagus tend to have more severe reflux disease than those with short-segment disease. Patients with long-segment Barrett's esophagus have a higher prevalence of hiatal hernia, more commonly have a defective lower esophageal sphincter, and dem-

onstrate greater esophageal acid and bilirubin exposure on 24-h pH and Bilitec monitoring[30,49]. Despite the differences in length, there is evidence that short and long-segment Barrett's esophagus are biochemically similar[48,50]. This is supported by the clinical observation that the risk of malignancy is similar for both short and long segments of Barrett's esophagus[51].

The presence of goblet cells is the *sine qua non* of Barrett's esophagus. The likelihood of finding intestinalization correlates with the length of the columnar segment. Once 4 cm of cardiac mucosa are present in the distal esophagus, nearly all patients will be found to have intestinal metaplasia on biopsy[49,52]. However, the location of goblet cells in a columnar-lined segment is not uniform, and often the entire length of columnar esophagus does not demonstrate intestinal metaplasia. Goblet cell density is greatest near the squamocolumnar junction and becomes more variable distally[29]. In other words, if intestinal metaplasia is present within a columnar-lined segment of the esophagus, it will always be present proximally at the squamocolumnar junction. Goblet cells might extend throughout the entire length of the columnar segment. The length of Barrett's esophagus is determined by the endoscopic length of columnar mucosa and not by the length of mucosa showing intestinal metaplasia. In other words, a 6-cm segment of columnar mucosa with intestinal metaplasia only at the proximal 1 cm is still considered long-segment Barrett's esophagus, but the clinical behavior of this long-segment Barrett's might differ substantially from a 6-cm segment of columnar mucosa with intestinal metaplasia throughout the entire length. The current definition of Barrett's esophagus does not take this into account.

The time course to develop goblet cells is uncertain, but it appears to take a minimum of 5-10 years[38,53]. Studies involving esophagectomy patients have indicated that cardiac mucosa develops rapidly, often within 1-2 years. Intestinalization of the columnar segment in these patients occurs significantly later, typically after another 3-5 years[18-20,33,54]. These findings might reflect an accelerated course of events because these patients often have significantly greater reflux of acid and bile than the typical patient with GERD. However, this clinically relevant human model does demonstrate the two-step process of Barrett's esophagus, starting with columnarization followed by intestinalization in some patients.

The molecular mechanisms by which cardiac mucosa acquires goblet cells remain to be elucidated. However, there is increasing evidence that expression of the homeobox gene Cdx-2 plays a pivotal role. The expression of this gene increases with progression from squamous mucosa with esophagitis to cardiac mucosa, and is maximal in the setting of intestinal metaplasia[55-57]. Experimental work has suggested that Cdx-2 expression can be modulated by the pH of luminal material[58]. Furthermore, an individual's response to an inflammatory stimulus might also participate in the mucosal adaptation to reflux disease. Fitzgerald *et al*[59] have demonstrated that esophagitis and Barrett's esophagus have distinct cytokine profiles that reflect different inflammatory responses to reflux-induced injury. Moreover, even within a given Barrett's esophagus segment, the inflammatory response is more severe at the proximal end near the squamocolumnar junction, which could explain the greater tendency for intestinalization to occur at this location[60]. Furthermore, the specific cytokine polymorphism of a given individual might also influence the development of Barrett's esophagus. Preliminary work from Gough *et al*[61], for example, has demonstrated that specific polymorphisms of interleukin (IL)-1 receptor antagonist and IL-10 are more common in patients with Barrett's esophagus than those with esophagitis. Thus, a genetically determined inflammatory response to reflux might influence the pathway of disease in each individual patient.

DYSPLASIA AND MALIGNANT TRANSFORMATION

Barrett's esophagus is a premalignant mucosa, and has an increased proliferation rate, decreased apoptosis, and an increased fraction of diploid and aneuploid cells compared to normal epithelium[13,62]. The combination of increased proliferation and decreased apoptosis allows genetic abnormalities to develop and accumulate, and drives the development of dysplasia and malignant transformation in Barrett's esophagus[63]. Although non-dysplastic Barrett's esophagus is a simple columnar epithelium with homogenous nuclei arranged close to the basement membrane, dysplasia results in both cytological and architectural abnormalities, including loss of nuclear polarity, pleomorphic appearance, and the development of glandular distortion[64]. By convention, there are four broad categories used by pathologists to describe the dysplastic process: (1) no dysplasia; (2) indefinite for dysplasia; (3) low-grade dysplasia; and (4) high-grade dysplasia. This classification system has been adapted for use in Barrett's esophagus from that used in ulcerative colitis[65,66]. The most significant category, high-grade dysplasia, is characterized by carcinoma *in situ* with malignant cells that do not invade the lamina propria.

The grading of dysplasia has great clinical utility in stratifying risk of subsequent cancer in patients with Barrett's esophagus, and to date, it is the most important predictive marker for the development of invasive adenocarcinoma. However, the ability to grade dysplasia remains a subjective endeavor, particularly outside specialized centers with expert gastrointestinal pathologists[67]. Even among focused gastrointestinal pathologists there is discordance, particularly with regard to the presence of low-grade dysplasia[68]. This lack of precision inherent in histopathological grading has stimulated efforts to identify more objective molecular and biochemical indicators of an increased risk for progression in patients with Barrett's esophagus. It has been demonstrated that in medically treated patients with Barrett's esophagus and low-grade dysplasia, the risk of progression is increased in patients with aneuploidy[69]. It is hoped that other molecular mark-

ers that are better able to predict which patients with Barrett's esophagus are at increased risk for progression will be identified in the future.

NATURAL HISTORY OF BARRETT'S ESOPHAGUS

Although it is widely accepted that Barrett's esophagus is a premalignant condition, the degree of risk remains uncertain. A meta-analysis by Shaheen *et al*[70] of 25 articles published between 1984 and 1998 concluded that the incidence of adenocarcinoma in patients with Barrett's esophagus was approximately 0.5% per patient-year, with a range from 0.2% to 2.9%. However, these studies were done in patients being treated for reflux, including those that had antireflux surgery, and thus these estimates might not reflect the true natural history of Barrett's esophagus progression. Known risk factors for progression to dysplasia and cancer include hiatal hernia size, the length of Barrett's esophagus, patient age, and the presence of cellular and molecular abnormalities, including abnormal ploidy status and *p16* or *p53* gene abnormalities[69,71-74].

The natural history of dysplasia is not well characterized, but the risk of malignancy increases with the development of low- and high-grade dysplasia. The best data have come from Reid *et al*[69], and in a carefully followed group of patients. they reported that low-grade dysplasia progressed to cancer in 4% over 5 years, whereas high-grade dysplasia led to cancer in 61% at 5 years. It is also clear that progression is variable, with some patients progressing at a steady pace over several years, while others have stable non-dysplastic or low-grade dysplasia in Barrett's esophagus for many years, and then rapidly develop high-grade dysplasia and cancer. Theisen *et al*[75] conducted a review of patients who received follow-up through the entire sequence of Barrett's esophagus, low-grade dysplasia, high-grade dysplasia, and adenocarcinoma to better understand the chronology of these events. In a group of 28 patients that presented with adenocarcinoma, a median of 24 mo passed from the initial diagnosis of Barrett's esophagus. Progression from low-grade to high-grade dysplasia occurred over a median of 11 mo. Once high-grade dysplasia was diagnosed, the median time to diagnosis of cancer was 3 mo. Although this timeline was variable for each individual, in the cohort of patients that had progression of Barrett's esophagus to cancer, the process occurred within 3 years. However, because most Barrett's esophagus patients do not progress onto dysplasia and cancer, the cohort in this retrospective study might not be applicable to all patients. Furthermore, because few of these patients had been in long-term Barrett's esophagus surveillance programs, it is not possible to separate prevalent from incident cancers in this group, and the actual month and year that Barrett's esophagus developed in each patient is also unknown. Thus, information on progression of Barrett's esophagus is largely anecdotal.

IMPACT OF ANTIREFLUX THERAPY ON THE NATURAL HISTORY OF BARRETT'S ESOPHAGUS

Medical therapy of Barrett's esophagus

There are three goals for treating patients with Barrett's esophagus: (1) stop reflux; (2) promote or induce healing or regression of the metaplastic epithelium such that the high-risk mucosa (intestinal metaplasia) is eliminated; and (3) halt progression to dysplasia and cancer. Most patients with Barrett's esophagus are treated medically; however, adequate medical therapy is difficult because of the degree of impairment of the lower esophageal sphincter and the poor esophageal body motility that are frequently present. This is likely to be the reason why the least controlled symptom in patients with Barrett's esophagus receiving medical treatment is regurgitation[76]. Medical treatment options are limited to dietary and lifestyle modifications, promotility agents, and acid-suppression therapy. Sampliner and the Practice Parameters Committee of the American College of Gastroenterology have stated that "the goal of therapy of Barrett's esophagus should be the control of the symptoms of GERD", and that "symptom relief is an appropriate endpoint for the therapy of Barrett's esophagus"[77]. However, this viewpoint flies in the face of logic. Gastroesophageal reflux causes both Barrett's esophagus and esophageal cancer. Symptoms are not part of the pathophysiology of the disease. Rather, they are merely the variably expressed byproduct of reflux. Many patients with Barrett's esophagus have few or no reflux symptoms; probably as a consequence of an altered sensitivity of the metaplastic epithelium to refluxed acid. Consequently, the eradication of symptoms, if present, cannot be equated with elimination of reflux. Katzka and Castell[78] have demonstrated that standard-dose omeprazole (20 mg/d) failed to suppress acid sufficiently to keep gastric pH neutral for a full 24 h in patients with Barrett's esophagus. Furthermore, increasing the dose of the omeprazole until all symptoms were alleviated was an unreliable measure of effective therapy, since 80% of patients studied with 24-h pH still had abnormal distal esophageal acid exposure[78]. Sampliner likewise found that high-dose proton pump inhibitor administration (lansoprazole, 60 mg/d) failed to normalize the 24-h pH test in over a third of patients with Barrett's esophagus who were tested while on therapy[76]. Even if complete suppression of acid could be achieved 24 h/d, 7 d/wk, for 350 d/year, impedance studies have shown that the number of reflux events is unchanged. Acid reflux events are merely converted to non- or weak acid reflux events, because the physiological abnormalities that lead to reflux are unaddressed by medical acid suppression therapy[79,80]. The role of continued weak or non-acid reflux in the progression of Barrett's esophagus is undefined, but it may explain the paucity of evidence that acid suppression therapy alters the natural history of Barrett's esophagus.

The second and third goals of therapy in patients with Barrett's esophagus are to eliminate the high-risk mucosa,

i.e. intestinal metaplasia, and prevent progression to dysplasia and cancer. Medical therapy has not been shown to achieve either of these goals reliably. Several reports have concluded that medical therapy does not cause regression of intestinal metaplasia[81-83]. This might be different in patients with short-segment Barrett's esophagus. Weston *et al*[84] have described the loss of goblet cells from lengths of intestinal metaplasia < 2 cm in 32% of patients treated medically for 1-3 years. In contrast, only two of 29 patients (7%) with lengths of intestinal metaplasia ⩾ 3 cm had loss of goblet cells.

With respect to the efficacy of medical therapy in preventing progression of Barrett's esophagus to dysplasia and cancer, there is speculation that prolonged, and perhaps inadequate acid suppression might actually promote the development of Barrett's esophagus and its complications[32]. Lagergren *et al*[85] have recently reported that the risk of esophageal adenocarcinoma was increased nearly eightfold among persons in whom heartburn, regurgitation, or both occurred at least once weekly compared to persons without these symptoms. They noted that the risk of esophageal adenocarcinoma was three times higher among patients who used medication for symptoms of reflux compared to those who did not use any antireflux medication[85]. Others, including Ortiz *et al*[82] and Hameeteman *et al*[86] have also linked medical therapy for Barrett's esophagus with progression to dysplasia and adenocarcinoma. In the study by Hameeteman *et al*[86] from the Netherlands, 50 patients with a columnar-lined esophagus were treated medically and followed from 1.5 to 14 years (mean 5.2 years). Of these 50 patients, initially only 34 had intestinal metaplasia on biopsy of the columnar mucosa. At completion of the study, 37 patients had intestinal metaplasia, which indicated that three patients developed Barrett's esophagus during the 5-year study period. In addition, at the start of the study, six patients had low-grade dysplasia and one had high-grade dysplasia. By the end of the 5-year study, 10 patients had low-grade dysplasia, three had high-grade dysplasia, and five had adenocarcinoma[86]. Similarly, Sharma *et al*[87] followed 32 medically treated patients with short segment Barrett's esophagus (mean length: 1.5 cm) for a mean of 36.9 mo, and found a 5.7% annual incidence of progression to dysplasia. During the 98 patient-years of follow-up in their series, two patients developed high-grade dysplasia, and one of these patients progressed to cancer. Recall that the expected rate of cancer is 1 per 100 patient-years of follow-up. All patients in the study by Sharma and colleagues were treated with omeprazole, ranitidine, and/or promotility agents. They commented that most patients developed dysplasia while on acid suppression medication, and they concluded that medical treatment does not prevent the development of dysplasia. A recent retrospective observational study in patients with Barrett's esophagus suggested that proton pump inhibitor use was associated with a reduced incidence of high-grade dysplasia or adenocarcinoma compared to patients not taking such medication, but there was no difference in the incidence of dysplasia between groups[88].

Antireflux surgery for Barrett's esophagus

In contrast to the ongoing weak or non-acid reflux that occurs with acid suppression therapy, antireflux surgery restores lower esophageal sphincter function and abolishes reflux of gastric contents into the esophagus. Consequently, an antireflux operation ends the repetitive injury to both the metaplastic and normal esophageal mucosa. Randomized clinical studies have confirmed superior control of reflux following antireflux surgery compared to medical therapy, and antireflux surgery has been proven safe, effective, and durable[82,89]. In addition, many patients are candidates for a minimally invasive laparoscopic approach associated with a short hospital stay and rapid recovery. We therefore favor the performance of an antireflux procedure in patients with Barrett's esophagus.

There have been conflicting reports about whether intestinal metaplasia regresses following antireflux surgery. Brand, in 1980, described complete regression in four of 10 patients with Barrett's esophagus who underwent fundoplication[90]. Subsequently, most reports have demonstrated that while some regression of the length of Barrett's esophagus is common, complete regression occurs only rarely, particularly with long-segment disease. In contrast, intestinal metaplasia of the cardia and short segments of Barrett's esophagus much more commonly regress to no intestinal metaplasia after fundoplication[91-93]. Furthermore, during prospective follow-up of patients with a columnar-lined esophagus without intestinal metaplasia treated either medically or with antireflux surgery, Oberg *et al*[94] showed that significantly fewer patients developed intestinal metaplasia after antireflux surgery.

Perhaps of greater importance is the issue of progression of Barrett's esophagus to dysplasia or cancer after surgical treatment of reflux disease. Compared to medical therapy, antireflux surgery is associated with a reduced incidence of dysplasia and adenocarcinoma. McCallum *et al*[95] have prospectively followed 181 patients with Barrett's esophagus. Twenty-nine had antireflux surgery while the remaining 152 patients were treated medically. After a mean follow-up of 62 mo in the surgical group and 49 mo in the medical group, there was a significant difference in the incidence of dysplasia and adenocarcinoma. Dysplasia was found in 3.4% of the surgical group compared with 19.7% in the medically treated group. No patient in the surgically treated group developed adenocarcinoma of the esophagus compared with two medically treated patients. They concluded that compared with medical therapy, an antireflux operation in patients with Barrett's esophagus was significantly associated with the prevention of dysplasia and cancer. Similarly, Katz *et al*[96] have followed 102 patients with Barrett's esophagus for a mean of 4.8 years. By 3 years, approximately 8% of the medically treated patients had developed dysplasia. In contrast, patients treated by antireflux surgery had a significantly reduced risk of developing dysplasia ($P = 0.03$)[96]. In the only randomized controlled trial that has compared medical therapy with antireflux surgery for Barrett's esophagus, Parrilla *et al*[97] showed that patients with functioning fundoplication had a significantly reduced incidence of developing dysplasia

compared to patients on medical therapy. Evidence at the molecular level has shown that antireflux surgery reduces the expression of genes potentially involved in the progression of Barrett's esophagus to cancer down to the level of control subjects without reflux[98,99]. These studies provide an insight into how antireflux surgery might be protective against progression of Barrett's esophagus to cancer.

Opposing these studies are two Swedish database studies that have suggested that antireflux surgery does not protect against progression to cancer. However, the serious flaw in both these studies is that the prevalence of Barrett's esophagus was not known in either population, and it is quite likely that far more patients in the antireflux surgery group had Barrett's esophagus than the comparison groups[100,101]. The presence of Barrett's esophagus is the leading known risk factor for subsequent development of esophageal adenocarcinoma, therefore, both studies only add to the controversy rather than provide any reliable answer to this important issue. Another factor that complicates any analysis of progression of Barrett's esophagus after antireflux surgery is that the cellular and genetic alterations that lead to the development of dysplasia and adenocarcinoma might have already occurred before the antireflux procedure. It has been estimated to take up to 6 years for adenocarcinoma to develop within Barrett's esophagus with low-grade dysplasia, and thus some cancers, particularly those that present during the first few postoperative years, probably do not represent progression of disease after surgery. McDonald *et al*[102] have made this point in a study from the Mayo Clinic. They found invasive adenocarcinoma in two patients and carcinoma *in situ* in one patient during surveillance after antireflux surgery, but they noted that no patient developed carcinoma after 39 mo, despite a median follow-up of 6.5 years, and a maximum follow-up of 18.2 years.

CONCLUSION

There is increasing evidence that at the normal gastroesophageal junction, esophageal squamous mucosa abuts oxyntic fundic mucosa of the stomach. With exposure to gastric juice, the squamous mucosa is injured, and over time becomes replaced by columnar cardiac mucosa. Deterioration of the lower esophageal sphincter allows reflux to extend up into the esophagus, and the squamocolumnar junction migrates proximally. Although it is likely that acidic gastric juice drives the transformation of squamous mucosa to cardiac mucosa, there is substantial evidence that other components of gastric juice, particularly bilirubin, are essential for subsequent intestinalization of the cardiac mucosa.

Barrett's esophagus is a premalignant mucosa, and the risk of malignant transformation is approximately 0.5% per patient-year. The finding of dysplasia is currently the most commonly used indicator of increased malignant risk, but it has high inter-observer variability. It is expected that ultimately molecular markers will prove more helpful than histology in Barrett's esophagus, and there are ongoing efforts to determine biomarkers that will better delineate an individual's risk for progression to cancer. Surveillance endoscopy in patients with Barrett's esophagus has proven efficacy, but is time-consuming and haphazardly applied. Currently, screening endoscopy is not recommended for Barrett's esophagus, but given the dramatic increase in the incidence of esophageal adenocarcinoma, new technologies that permit widespread and cost-effective screening are needed. Patients with Barrett's esophagus are commonly treated with acid-suppressive medication, but there are few data that this therapy alters the natural history of the disease, and thus current medical guidelines are to treat for symptomatic relief rather than for documented pH control. Antireflux surgery abolishes reflux and has been shown to normalize gene expression in patients with Barrett's esophagus, but controversy persists regarding the impact of an antireflux procedure on the risk of Barrett's esophagus progression.

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TOPIC HIGHLIGHT

Marco G Patti, MD, Professor, Director, *Series Editor*

Effect of medical and surgical treatment of Barrett's metaplasia

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Abstract

Barrett's esophagus (BE) is a change in the esophageal mucosa as a result of long-standing gastroesophageal reflux disease. The importance of BE is that it is the main risk factor for the development of esophageal adenocarcinoma, whose incidence is currently growing faster than any other cancer in the Western world. The aim of this review was to compare the common treatment modalities of BE, with the focus on proton pump inhibitors and operative fundoplication. We performed a literature search on medical and surgical treatment of BE to determine eligible studies for this review. Studies on medical and surgical treatment of BE are discussed with regard to treatment effect on progression and regression of disease. Although there is some evidence for control of reflux with either medical or surgical therapy, there is no definitive evidence that either treatment modality decreases the risk of progression to dysplasia or cancer. Even though there is a trend toward antireflux surgery being superior, there are no definitive studies to prove this.

Key words: Barrett's esophagus; Intestinal metaplasia; Proton pump inhibitors; Nissen fundoplication; Esophageal adenocarcinoma; Gastroesophageal reflux

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INTRODUCTION

Barrett's esophagus (BE) is defined as a change of any length in the distal esophageal epithelium, which can be recognized as columnar-type mucosa at endoscopy and confirmed as intestinal metaplasia (IM) by biopsy of the tubular esophagus[1]. BE is a complication of gastroesophageal reflux disease (GERD) through damage of the esophageal mucosa from refluxed contents[2,3]. It is thought to be present in around 10% of patients with GERD[3,4], although the exact incidence is unknown. As a result of the substantial increase of esophageal adenocarcinoma (AC) in patients with BE, it is considered the major risk factor for this form of cancer. In fact, over the past decade there has been acceleration in the incidence of AC in the Western world, presumably from a rise in GERD, its treatment, or other environmental factors. In the United States, it is estimated that 1.5-2 million people have BE[5].

It has been estimated that the risk for developing esophageal AC when IM is present is approximately 0.5% per year[6]. Although the factors that affect progression are not completely known, it is tempting to assume that the risk is increased by continued exposure of the IM to gastric contents[7].

Screening for BE in patients with chronic heartburn is not widely considered to be cost-effective, but surveillance in patients with BE is generally advised[8]. This, however, puts a heavy burden on resources for endoscopists. To prevent the development of esophageal cancer and to try and reduce the need for surveillance, the available treatment modalities for BE have been evaluated. The goal for treating patients with BE is generally directed at controlling associated symptoms of GERD, because quelling symptoms is a much more immediate endpoint for adjusting or changing therapy. Nevertheless, in this review, we discuss the possible treatment options for BE, with a focus on their effect on the Barrett's mucosa itself. The two most common treatments of GERD and associated BE are medical (proton pump inhibitors, PPIs) and surgery (fundoplication). Recently, more attention has been paid to other possible (medical) treatment options of BE that are not specifically aimed at reducing reflux. We briefly cover these treatment options as well.

LITERATURE SEARCH

A PubMed search was performed to identify publications using the following MeSH terms: "Barrett esophagus" and "proton pump inhibitors" or "surgical procedures, operative". Publications had to be published in the English language in peer-reviewed journals. Only studies published from 2000 onward with endoscopic biopsy results after treatment were deemed eligible. If publications were from the same research group, the most recent or most applicable study was chosen.

The abstracts of the results were read to determine eligibility for this review. If deemed eligible, full-text versions of the studies were acquired. From these full-text articles, references were checked to find publications that were missed using the search with MeSH terms[9-12]. Twenty studies were found to be eligible for this review. Five were on medical treatment (PPIs), 11 were on surgical treatment and four compared the two treatments.

DEFINITIONS

Progression of BE in this review is defined as a change in histological findings on biopsy from either IM to any form of dysplasia or an increase in grade of dysplasia. Development of AC is also considered progression of disease. Regression is defined as change from high-grade dysplasia (HGD) to low-grade dysplasia (LGD) or no dysplasia, change from LGD to metaplasia or loss of metaplasia, and change from IM to complete loss of metaplasia. Shortening of the segment or development of squamous cell islands, although considered by some as regression, usually is not accurately measured and reported, and is therefore, not considered regression in our report. Short-segment BE (SSBE) is defined as a length $\leqslant$ 3 cm seen at endoscopy and confirmed by biopsy. Long-segment BE (LSBE) is defined as > 3 cm.

LIMITING PROGRESSION

Ultimately, the goal of treatment for BE is to prevent cancer. Both medical and surgical treatment studies therefore have traditionally been focused on showing results of preventing progression of disease. We first discuss the results for PPI treatment, then those of operative treatment using fundoplication, and finally, studies that have compared these two treatment modalities.

Medical treatment

Three recent studies have investigated the effect of PPI treatment on the risk of progression of BE to dysplasia or AC[9,13,14]. The results of studies of PPI treatment with regard to progression and regression of disease are shown in Table 1. The results of these studies suggest a protective effect of PPIs in limiting the progression of BE.

In the study by Hillman *et al*[13], (350 patients with BE over a 20-year period), patients were stratified according to delay in starting PPI therapy after the diagnosis of BE was established. Patients who delayed PPI therapy for $\geqslant$ 2 years after being diagnosed with BE had 5.6 times higher risk of developing LGD than patients who used PPI within the first year after diagnosis. Furthermore, patients with BE had up to a 20 times higher risk of developing HGD or AC when PPI therapy was delayed for 2 years after diagnosis of BE. Although this suggests a substantial protective effect, the absolute risk of developing HGD or AC was low (n = 11; 3%) at a median follow-up of 4.7 years.

The small rate of progression of BE makes it very difficult to show a difference between treatments. In another study, the risk of developing LGD within 5 years of the diagnosis of BE was around 2.5%, and the risk of HGD/AC was around 2% while taking PPI therapy. Cooper *et al*[9] have shown this in a study of 188 patients with IM who were treated with a PPI. However, when following patients for > 5 years, Nguyen *et al*[14] recently have found a much higher risk of developing AC. They have studied 344 patients diagnosed with BE without dysplasia, with a mean follow-up of 7.6 years. They found that the chance of developing HGD or AC was 7.4%. Moreover, this risk was even higher when not taking PPIs (14.2%). Taken together, the results of these non- controlled studies suggest that PPIs have a protective effect, but they do not eliminate the risk of developing AC.

Surgical treatment

Surgical treatment of BE most often involves fundoplication for GERD. Where PPIs are only able to decrease acid content in the stomach (and thus change the pH of the refluxate), surgery has the ability to prevent any type of reflux. Therefore, many have argued that surgery is a more effective therapy for BE. All 11 publications on surgical treatment for BE that met our screening criteria included results on prevention of progression, as well as regression of metaplasia or dysplasia[15-25]. In this section, we discuss only the results of the effect of fundoplication on the rate

Table 1 Medical therapy and surgery for limiting progression and causing regression of Barrett's esophagus *n* (%)

Publication	No. of patients	Follow-up (yr)	Adenocarcinoma	Dysplasia	Regression
Medical therapy					
Hillman *et al*[13], 2004	279	4.7	7 (2.5)	5 (1.8)	NA
Cooper *et al*[9], 2006	188	5.1	3 (1.6)	6 (3.2)	NA
Nguyen *et al*[14], 2009	231	7.6	17 (7.4)	53 (23)	NA
Heath *et al*[10], 2007	82	0.9	6 (7.3)	9 (11)	34 (41)
Horwhat *et al*[11], 2007	67	3.8	2 (3.0)	21 (31)	13 (19)
Total	847	4.4	35 (4.1)	94 (11.1)	47 (31.5)
Surgery					
Hofstetter *et al*[15], 2001	79	5.0	0	4 (5)	16 (20)
Bowers *et al*[16], 2002	64	4.6	0	1 (2)	31 (48)
Mabrut *et al*[17], 2003	13	3.8	0	0	6 (46)
Oelschlager *et al*[18], 2003	90	2.6	1 (1)	3 (3)	30 (33)
Desai *et al*[19], 2003	50	3.1	0	1 (2)	9 (18)
O'Riordan *et al*[20], 2004	57	3.8	2 (4)	2 (4)	14 (25)
Abbas *et al*[21], 2004	33	1.5	1 (3)	2 (6)	13 (39)
Zaninotto *et al*[22], 2005	35	2.3	0	0	6 (17)
Ozmen *et al*[23], 2006	37	1.6	0	1 (3)	6 (16)
Biertho *et al*[24], 2007	70	4.2	0	3 (4)	23 (33)
Biertho *et al*[25], 2009	23	4.5	0	0	14 (61)
Total	551	3.4	4 (0.7)	17 (3.4)	168 (30.5)

NA: Not applicable.

of progression. The results of studies on surgical treatment for limiting progression and causing regression are summarized in Table 1.

In the reported case series, the number of patients is relatively low since a minority of patients is referred for surgery. As a result, because progression can take a long time and is still a relatively rare event (especially on medical therapy), large studies with several hundred patients would be needed to show a clinically significant benefit. Still, it is interesting to look at several trends, and as can be seen in Table 1, almost uniformly there is a low incidence of progression to dysplasia and even a lower incidence to AC.

Hofstetter *et al*[15] have published the study with the longest follow-up. They showed results for a series of 97 patients, with complete endoscopic follow-up in 79, at a median of 5 years. No patients developed HGD or AC, but four had progression of metaplasia to LGD (5%). Bowers *et al*[16], have reported a similar series with a mean follow-up of 4.6 years. Their 104 patients underwent open or laparoscopic fundoplication. Of these, 64 patients had endoscopic follow-up with biopsy. None of the patients developed HGD or AC. Only one patient had progression to LGD (1.5%).

Control of reflux

The hypothesis that surgery is superior to medical therapy comes from the assumption that surgery provides better control of GERD than do PPIs, and this should translate into lower progression rates. Indeed, there is some circumstantial evidence for this. Lagergren *et al*[26] and Csendes *et al*[27] have suggested that, when esophageal AC occurs after antireflux surgery, it is usually in the face of persistent or recurrent reflux. This observation, that control of reflux is essential in preventing progression of disease, is backed up by the fact that, in most studies, the patients with progression after surgical treatment seem to have recurrent reflux. In a series of 58 patients by O'Riordan *et al*[20] who underwent open or laparoscopic Rossetti-Nissen fundoplication, four were found to have progression of disease after a follow-up of 45 mo. All four patients were found to have abnormal postoperative acid scores[20]. In another study, Biertho *et al*[24] have published the results of 70 patients with BE who had endoscopic follow-up for 4.2 years after laparoscopic fundoplication. Three patients had progression of disease, but none developed HGD or AC. All three patients with progression had recurrence of GERD symptoms. We published our results of 106 patients with BE who underwent laparoscopic fundoplication[18]. Endoscopic follow-up with biopsies was performed in 90 patients with a median follow-up of 30 mo. One patient was found to have developed AC at 10 mo after the operation (and thus likely had at least dysplasia at the time of operation). One patient developed HGD and one LGD. The patient with HGD had LGD preoperatively and for 3 years thereafter, and then developed recurrent GERD symptoms with an abnormal 24-h pH. One year later this patient was found to have developed HGD despite being on medical therapy. Still, despite the fact that surgery is not perfect, the rate of progression to HGD or AC seems around 1.5%, which is lower than that typically seen in medical treatment.

One of the difficulties in evaluating the results of these treatments is the overall low incidence of patients with BE progressing to AC. Although decreasing the total burden of BE might actually decrease the risk of cancer, it is difficult to track. The results of the studies suggest that surveillance after medical treatment is necessary. After surgical treatment, there is also still progression of

Table 2 Medical therapy *vs* surgery for Barrett's esophagus *n* (%)

Publication	Treatments	PPI	Nissen	Progression PPI	Progression Nissen	Regression PPI	Regression Nissen	Study type
Gatenby *et al*[6], 2009	PPI *vs* Nissen	646	41	154 (24)	4 (10)	NA	NA	Cohort
Parrilla *et al*[28], 2003	H2RA/PPI *vs* Nissen	43	58	10 (23)	5 (9)	2 (5)	5 (9)	RCT
Rossi *et al*[29], 2006	PPI *vs* successful Nissen	19	16	NA	NA	12 (63)	16 (100)	Case comparison
Total		708	115	164 (23.8)	9 (9.1)	14 (22.6)	21 (28.4)	

PPI: Proton pump inhibitor; H2RA: H2 receptor antagonist; RCT: Randomized controlled trial; NA: Not applicable.

disease (particularly in patients with LSBE), although the risk seems to become very small when this treatment is successful. Patients are generally reluctant to have surveillance, as shown by the low number of patients who actually have endoscopy after fundoplication. Another difficulty in interpreting the results is the follow-up of these studies that ranges from 0.9 to 7.6 years. With a disease that, in general, progresses only slowly, studies with follow-up of 10-20 years are needed. In contrast, studies on surgical treatment with the longest follow-up have still shown very low incidence of progression. The study on medical treatment with the longest follow-up did show a higher chance of progression of disease[14], although that study was possibly confounded by selection bias.

Medical vs surgical treatment

There have been very few studies comparing medical and surgical therapy; in fact, in our review, we only found two studies on progression of disease worthy of comment. The results of these are summarized in Table 2.

In one, Gatenby *et al*[6] published the results of their review of a cohort of 738 patients with BE enrolled in a national registry. They compared patients with anti-reflux surgery (n = 41) to those treated medically with PPIs (n = 551), H2 receptor antagonists (H2RAs) (n = 42), H2RA followed by PPI (n = 95), or no treatment (n = 9). Their outcome parameters were progression of disease to LGD, HGD or AC. They could not control for many other selection factors, which might have confounded the results, such as severity of disease. After a follow-up of 5 years after medical therapy and 6 years after surgical therapy, there was however a trend toward antireflux surgery being more protective. No patients in the antireflux group developed HGD or AC as compared to 4.3% in the all-medical therapies group (P = 0.13). There were not enough patients in the surgical arm to determine if this was a significant difference.

Parrilla *et al*[28] have published the only randomized study comparing medical treatment (n = 43) and antireflux surgery (n = 58). In that study, 101 patients with BE were treated between 1982 and 2000. Medical treatment consisted of H2RA treatment initially and then omeprazole from 1992 onward. Surgery was performed through laparotomy with Nissen fundoplication in 56 patients and a Collis-Nissen procedure in the other two because of short esophagus.

All patients had annual clinical, endoscopic and histological follow-up, and patients who had an operation also had a pH study and manometry at 1 year postoperatively and every 5 years thereafter, or if they presented with recurrent GERD symptoms. Mean follow-up was 6 years for the medical therapy group and 7 years for the surgical group. Progression of BE to any dysplasia was found in eight patients (19%) in the medical treatment group and in three in the surgical group (5%). Although the P value was not specified in their paper, according to our calculations using Fisher exact test, there was a protective effect of fundoplication (P = 0.05). Two patients in each group progressed to AC, which was confirmed after esophageal resection. Although differences in progression rates between the two groups were not significant according to the authors, when a sub-analysis was performed including only patients in the surgical arm with normal pH, the progression rate dropped to 2%, which was a significantly lower chance of progression of disease than in the medical group (P < 0.05).

CAUSING REGRESSION

IM without dysplasia is a benign condition, therefore, inducing regression is not considered as important as limiting progression. Nevertheless, if IM is no longer present, then it theoretically can no longer progress to cancer, thus it has been reported as a surrogate for measuring the response of various therapies. Disappearance of IM seems to be a slightly more common occurrence after effective treatment of GERD and therefore is a more easily studied endpoint.

Medical treatment

The only two studies that we found that have published results of regression of BE following medical treatment are by Heath *et al*[10] and Horwhat *et al*[11]. The results of these studies are shown in Table 1, together with the studies on progression of disease.

The purpose of the study by Heath *et al*[10] was to investigate the effect of long-term celecoxib in patients with BE with dysplasia. The mechanism for chemoprevention of celecoxib is thought to be through inhibition of cyclooxygenase (COX)[30]. They randomized 100 patients with low or high-grade Barrett's dysplasia to treatment with either celecoxib (n = 49) or placebo (n = 51). Although this study did not focus on PPI therapy, > 90% of these patients were concomitantly on a PPI. After 48 wk of treatment, endoscopic biopsy results showed a regression of dysplasia in 41.9% of patients on celecoxib and 41%

on placebo (P = 0.89), either from LGD to no dysplasia or from HGD to LGD (although differentiation between those events in this study was not possible). In contrast, 14% (n = 6) and 15.4% (n = 6) respectively had an increase in highest grade of pathology, with three patients in each group developing AC. These mixed results might say more about the variability in interobserver reliability of dysplasia, as has been reported[31]. However, the results do suggest that patients with dysplasia can regress with medical therapy alone.

Horwhat *et al*[11] looked at LSBE and SSBE. They contacted 101 patients after a mean follow-up of 46 mo. Most patients received PPI therapy but seven underwent fundoplication. Of the 38 patients with LSBE, 23 underwent endoscopy. Six patients developed dysplasia (26%) and two cancer (9%). No patient with LSBE had regression of disease. Of the 63 patients in the SSBE group, 44 underwent endoscopy. Three patients were found to have progression of disease (7%) *vs* 13 with regression (30%). They found an almost linear relationship between BE segment length and normalization of the epithelium, that is, the chance of progression of disease is significantly higher in LSBE compared with SSBE. Unfortunately, it is unclear in this study whether the patients with regression or progression had medical or surgical treatment.

Surgical treatment

The results of regression of BE with surgical treatment are shown in Table 1, together with the results of progression. The literature suggests that regression of BE occurs with some regularity after fundoplication, even regression to completely normal squamous epithelium. Hofstetter *et al*[15] have reported that 16 of their 79 patients (20%) had regression of disease in some fashion. Of the 16 patients with LGD, seven had regression (44%), and of the 63 patients with IM, nine had complete loss of metaplasia (14%).

It is important to consider that LGD is sometimes over-reported because of inflammation from ongoing GERD, and surgery could make it easier for the pathologist to interpret the biopsies. Nevertheless, other studies have suggested regression in a substantial number of BE patients. Desai *et al*[19] have found a loss of metaplasia in seven of 50 patients (14%) postoperatively. Two out of the three patients with LGD had regression to non-dysplastic BE. In the study by Bowers *et al*[16], it has been found that 31 of 66 patients had loss of IM (47%) after antireflux surgery. Patients with regression had shorter lengths of BE preoperatively and longer follow-up after the operation.

That patients with SSBE have a higher incidence of regression than those with LSBE seems logical, and it has been consistently seen in studies where long and short-segment BE has been distinguished. In the study by O'Riordan *et al*[20], eight of 57 patients (14%) were found to have complete regression. Six of these patients had SSBE preoperatively. They have also found regression from LGD to non-dysplastic BE in six of eight patients. Biertho *et al*[24] have reported that complete regression was found in 23 of their 70 patients (33%). All patients with regression had SSBE preoperatively. Regression from LGD to non-dysplastic BE occurred in two of three patients.

Our experience mirrors that of other authors who have found that complete regression occurs only in patients with SSBE. Of the 54 patients with SSBE before surgery, 30 (54%) had no evidence of IM at last follow-up. In contrast, none of the 38 patients with LSBE before surgery had complete regression[18]. These observations suggest that the chance of accomplishing regression is especially high in patients with earlier disease. Therefore, earlier referral for surgery might increase the chance of cure from BE even further.

Medical vs surgical treatment

Only one small study comparing medical and surgical treatment directly has been published that focuses on regression of BE. The results are summarized in Table 2.

Rossi *et al*[29] prospectively studied 19 patients with high-dose PPI and 16 patients with fundoplication. All patients had LGD. After 18 mo follow-up, a high percentage of patients were found to have regressed to IM after medical (63%) as well as surgical treatment (100%). Although the rate was higher in the surgical group, the small numbers make it difficult to use the study to draw any definitive conclusions. Parrilla *et al*[28] also have reported data on regression of disease in their randomized study, although they do not comment on this, with 2/43 (4.6%) having regression from LGD to IM with medical therapy, and 5/58 (8.6%) after surgical therapy ($P > 0.05$).

When comparing both treatment modalities, antireflux surgery seems to be more successful in prevention of progression and in promoting regression than medical treatment with PPI. The number of patients studied and the quality of the studies however were low, therefore, a firm conclusion cannot be drawn. Complications from the operation are also not taken into account and these studies generally come from surgical centers of excellence. On the other hand, the patients that underwent an operation are more likely to have had more severe disease than the patients that are treated medically.

OTHER MEDICAL TREATMENT

Almost all patients with BE, because of their associated GERD, are treated with PPIs (unless they have surgery), therefore, it makes sense to evaluate the effect of acid reduction on the natural history of BE. However, there have been other medical therapies investigated for the purpose of addressing IM primarily. For example, Vaughan *et al*[32] have shown a potential role for nonsteroidal anti-inflammatory drugs (NSAIDs). The effect of NSAIDs is thought to be through their anti-inflammatory effect through inhibition of COX-2 production[33]. Ogunwobi *et al*[34] have made a theoretical argument for statins, stating that they might affect proliferation and apoptosis in esophageal cancer cells. The protective effect of these medications is further supported by a

recent study by Nguyen *et al*[35]. In this retrospective observational study using pharmacy data, they have shown a reduced risk of developing AC in patients with BE and filled NSAID prescriptions. They have also studied statins as chemopreventive medications, however, they are concerned about confounding with statin therapy because patients had short periods of use, therefore, conclusions cannot be drawn about these medications.

Other publications contradict the role of NSAIDs in preventing progression. One is the study by Heath *et al*[10] that was discussed earlier, which did not find a difference when comparing patients on or off celecoxib. Gatenby *et al*[36] have published results of a national registry in the United Kingdom of BE, where they did not find a difference in development of dysplasia or AC between patients on or off aspirin. To evaluate further the effect of aspirin treatment of BE on progression to cancer, a large randomized trial (AsPECT) is ongoing, which is comparing patients on PPI therapy with and without aspirin[37].

Many other medications, such as ursodeoxycholic acid, hormone replacement therapy and n-3 fatty acids have been studied[38-41], but all have too little information to recommend their use currently. Dietary interventions through antioxidants, fiber and vitamins have been studied for their effect on risk of cancer in general and for prevention of esophageal AC. However, mixed results have been reported[42].

Very few clinical studies have been carried out on treatment modalities other than antireflux surgery using fundoplication, or medical treatment using PPIs. Therefore more (large) studies are necessary before any firm conclusions can be drawn on the chemopreventive qualities of agents such as aspirin, selective COX inhibitors or diet modifications.

CONCLUSION

Consensus on the best treatment for BE remains elusive, because there has not been a large definitive study to date that has compared PPIs and fundoplication (nor is there likely to be one). There is, however, a trend toward lower risk of progression with anti-reflux surgery compared with anti-acid medication, especially when anti-reflux surgery is successful. In addition, there seems to be a greater chance of regression of disease with anti-reflux treatment, but the importance of this regression is unclear. Theoretically, surgery controls gastroesophageal reflux better than PPIs do (which mostly reduces the acid component), therefore, it is appealing for some to consider this a real difference, and therefore, recommend surgery for patients with BE, even though it is not definitively proven. As a result, treatment of BE has to be given based on the patient's preference and control of GERD symptoms. Just like GERD without IM, those with IM should consider fundoplication if symptomatic, despite appropriate medical therapy. The effect of fundoplication on the natural history of the epithelium should be a secondary concern. Whichever treatment is pursued, surveillance remains important, because the risk of cancer is not eliminated despite the decrease in risk through both PPIs and surgery.

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TOPIC HIGHLIGHT

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Endoscopic treatment of Barrett's esophagus: From metaplasia to intramucosal carcinoma

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Abstract

The annual incidence of adenocarcinoma arising from Barrett's esophagus (BE) is approximately 0.5%. Through a process of gradual transformation from low-grade dysplasia to high-grade dysplasia (HGD), adenocarcinoma can develop in the setting of BE. The clinical importance of appropriate identification and treatment of BE in its various stages, from intestinal metaplasia to intramucosal carcinoma (IMC) hinges on the dramatically different prognostic status between early neoplasia and more advanced stages. Once a patient has symptoms of adenocarcinoma, there is usually locally advanced disease with an approximate 5-year survival rate of about 20%. Esophagectomy has been the gold standard treatment for BE with HGD, due to the suspected risk of harboring occult invasive carcinoma, which was traditionally estimated to be as high as 40%. In recent years, the paradigm of BE early neoplasia management has recently evolved, and endoscopic therapies (endoscopic mucosal resection, radiofrequency ablation, and cryotherapy) have entered the clinical forefront as acceptable non-surgical alternatives for HGD and IMC. The goal of endoscopic therapy for HGD or IMC is to ablate all BE epithelium (both dysplastic and non-dysplastic) due to risk of synchronous/metachronous lesion development in the remaining BE segment.

Key words: Barrett's esophagus; High-grade dysplasia; Intramucosal carcinoma; Esophagectomy; Endoscopic mucosal resection; Radiofrequency ablation; Cryotherapy

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INTRODUCTION

The annual incidence of adenocarcinoma arising from Barrett's esophagus (BE) is approximately 0.5%[1-3]. Through a process of gradual transformation from low-grade dysplasia (LGD) to high-grade dysplasia (HGD), adenocarcinoma can develop in the setting of BE[4]. The clinical importance of appropriate identification and treatment of BE in its various stages, from intestinal metaplasia (IM) to intramucosal carcinoma (IMC) hinges on the dramatically different prognostic status between early neoplasia and more advanced stages. Once a patient has symptoms from adenocarcinoma, there is usually locally advanced disease with an approximate 5-year survival rate of about 20%[5,6].

Esophagectomy has been the gold standard treatment for BE with HGD, due to the suspected risk of harboring occult invasive carcinoma, which has been

estimated to be as high as 40%[7,8]. Our previous analysis of the published literature demonstrated that the true prevalence of submucosal invasive carcinoma in the setting of HGD was actually 12%, which was much lower than the pooled reported historical rate of 40%[9]. Esophagectomy has also been routinely performed for BE with IMC, despite a low incidence of lymph node metastasis of < 1% that is associated with non-invasive T1a disease[10]. Additionally, esophagectomy is associated with significant morbidity and mortality even in high-volume centers[11,12].

With these issues in mind, the paradigm of BE early neoplasia management has recently evolved, and endoscopic therapies have entered the clinical forefront as acceptable non-surgical alternatives for HGD and IMC. The goal of endoscopic therapy for HGD or IMC is to ablate all BE epithelium (both dysplastic and non-dysplastic) due to risk of synchronous/metachronous lesion development in the remaining BE segment[10]. Endoscopic therapies can be further subdivided into tissue-acquiring and non-tissue-acquiring modalities. Tissue acquisition can be achieved through endoscopic mucosal resection (EMR), while photodynamic therapy (PDT), radiofrequency ablation (RFA), and cryotherapy all ablate tissue without the benefit of histological specimen retrieval. A brief technical review and pertinent available efficacy/safety data are summarized for these various modalities in treating stages of early BE neoplasia that ranges from IM to IMC. Modalities such as argon plasma coagulation, multipolar electrocoagulation, and laser therapies are not be discussed as current mainstay therapies due to high BE relapse rates, infrequent usage, or significant risk of buried gland development[13].

EMR

EMR can be performed through a variety of techniques: free-hand, lift-and-cut, cap-assisted, or band-assisted. Injection of saline with a sclerotherapy needle is performed to create a submucosal fluid cushion, and a snare is used to entrap directly the mucosal tissue in the free-hand method. In the lift-and-cut approach, a dual channel endoscope is used to introduce simultaneously a grasping forceps and snare for resection. The cap technique uses a clear distal attachment with an inner rim around which a crescent-shaped snare is carefully fitted. The target area is injected for submucosal lift, then suction is applied through the cap, and tissue is entrapped by the snare for subsequent mucosal excision. Band-assisted techniques are modifications of the variceal band ligation device that allows for injection and then deployment of bands for mucosal pseudopolyp creation. A snare is then introduced and the mucosa is resected either above or below the band[14].

Focal EMR can be performed for endoscopically visible lesions that are suspicious for malignancy. However, several previously published studies on focal resection have demonstrated a high rate of synchronous and recurrent lesion development, which ranged from 14% to 47%, and increased with longer observation times[15-22]. As a result of this limitation of focal EMR, complete Barrett's eradication EMR (CBE-EMR) has been advocated and performed in select centers, with the intent to remove all BE epithelium curatively, to reduce the potential risk of synchronous or metachronous lesion development. Complete responses have ranged from 76% to 100%. The complication profile of EMR includes stricture formation, with an incidence rate that approaches 50%, bleeding and perforation. Of note, most esophageal stenoses and bleeding are amenable to endoscopic treatment[23-26].

When evaluating the effect of EMR on final histopathological staging, our center long-term results with CBE-EMR have revealed that initial EMR upstaged seven of 49 (14%) and down-staged 15 of 49 (31%) final pathology results when compared to pre-EMR biopsy results. Among the upstaged group, four patients had advanced pathology that was found after index EMR (either submucosal carcinoma or IMC with lymphatic channel invasion). All four of these patients had visible lesions upon endoscopy[26]. This is the crucial point that distinguishes EMR from all other non-tissue-acquiring modalities that would have inadvertently attempted ablation of advanced pathology in the setting of presumed BE HGD treatment.

PDT

The goal of PDT is destruction of tissue through a light-sensitizing reaction sequence. A photosensitizer is first administered which accumulates in esophageal malignant and pre-malignant tissue before light activation therapy. Porfimer sodium is the most common photosensitizer, and this is delivered intravenously 72 h before the procedure. Alternatively, oral 5-aminolevulinic acid (ALA) and intravenous m-tetrahydroxyphenyl chlorine (mTHPC) can be used. Activation of the photosensitizing agent occurs upon exposure to either bare cylinder or balloon-based diffusing light fibers that are placed alongside the target tissue via an endoscopic approach. The resulting molecular excitation reacts with oxygen to create radical oxygen species that cause eventual cell apoptosis[27].

A multicenter trial by Overholt *et al*[28] randomized BE HGD patients to receive twice daily oral omeprazole (20 mg) with or without porfimer sodium PDT administration. The study found that, at 5 years, PDT was significantly more effective than proton pump inhibition (PPI) alone, in elimination of HGD (77% *vs* 39%, $P < 0.0001$). Prevention of cancer progression was a secondary outcome that also showed a significant difference, with the PDT/PPI group demonstrating half the likelihood of developing cancer and longer time to cancer progression.

Overholt *et al*[29] have conducted another porfimer PDT study of 103 patients with LGD, HGD, or IMC with a mean follow-up of 50.65 mo (SD 20.57) (range: 2-122 mo). Intention to treat success rates were 92.9%,

77.5%, and 44.4% for the respective LGD, HGD, and IMC groups. Three patients (4.6%) developed sub-squamous adenocarcinoma. Strictures occurred in 18% with one session of PDT, 50% with two treatments, and 30% in the overall group.

ALA PDT has shown 97% and 100% complete response rates for treatment of BE with HGD and IMC, respectively, in a median follow-up period of 37 mo (interquartile range: 23-55 mo). Disease-free survival of HGD patients was 89%, and 68% in patients with IMC. The calculated 5-year survival was 97% for HGD and 80% for IMC, but no deaths were related to Barrett's neoplasia[30].

In a pilot study of PDT using mTHPC for seven patients with HGD and 12 patients with IMC, Lovat and colleagues found that treatment results were variable based on red versus green light usage. Successful ablation was achieved in four out of six mucosal carcinoma and three out of four HGD patients who received red light. However green light exposure failed to achieve successful disease eradication or long-term remission[31]. Significant complications such as death occurred after premature biopsy performance after treatment. This limited sample size study demonstrated that although mTHPC can destroy BE epithelium, the optimal light and drug dosimetry are still unknown[31].

To date, no randomized, controlled prospective trials have been conducted to compare PDT and surgery for BE neoplasia management. However, a retrospective data analysis of HGD patients who received PDT (n = 129) or esophagectomy (n = 70) has revealed no statistically significant differences in mortality or long-term survival based on choice of treatment modality[32].

The major side effects of PDT include photosensitivity that requires patients to avoid post-procedure skin sunlight exposure, non-cardiac chest pain, and symptomatic stricture formation. Risk factors for post-PDT stricture development include history of prior esophageal stricture, performance of EMR before PDT, and more than one PDT treatment in a single session[33]. Another concern about PDT is development of sub-squamous BE glands that could harbor neoplastic potential. The clinical significance of this finding is still not fully understood. However, reports of adenocarcinoma arising from sub-squamous BE glands after PDT therapy have been described[29,34]. For these reasons, PDT usage has gone out of favor in recent years, with the availability of other endoscopic ablative options.

RFA

Using a either a balloon-based catheter or a focal device, RFA of BE tissue can be achieved in either a circumferential or localized fashion. After initial insertion of a sizing balloon into the esophagus, the optimal size of the circumferential balloon is selected based on various pressure measurements in different locations. The ablation process is a series of two separate applications of direct thermal energy with the electrodes embedded in either the circumferential or focal device. Scraping of treated tissue is performed between the first and second ablation to ensure adequate and uniform thermal contact. The most common complications associated with RFA include non-cardiac chest pain, non-transmural lacerations, and stricture formation (lower stricture rate when compared to EMR).

After thermal dose-escalation animal testing and pre-esophagectomy human experiments[35,36], the first larger clinical evaluation of RFA was performed on BE patients without dysplasia in the Ablation of Intestinal Metaplasia (AIM) study from 2003 to 2005. This multicenter trial demonstrated a 70% complete remission of BE in the circumferential-balloon-treated group at 1 year follow-up, without evidence of subsequent stricture formation or buried BE among 4306 biopsy fragments evaluated[37]. A subsequent AIM Ⅱ study reported 98% complete remission of IM after stepwise circumferential therapy with additional focal ablative therapy of remaining BE[38].

RFA was also studied in 142 patients with BE HGD. At 1 year follow-up, complete remission of HGD was achieved in 90.2%, complete remission of dysplasia in 80.4%, and complete remission of IM in 54.3% of patients[39]. In a recent landmark multicenter, sham-controlled trial, 127 patients with dysplastic BE were randomly assigned to receive either RFA or a sham procedure. The measured primary outcomes at 1 year included complete eradication of dysplasia and intestinal metaplasia. Based on an intention-to-treat analysis, in patients with LGD, complete eradication of dysplasia occurred in 90.5% in the ablation group, compared to only 22.7% in the control group ($P < 0.001$). In the HGD sub-group, complete eradication occurred in 81% of ablated patients as compared with 19% of the control group ($P < 0.001$). Overall, 77.4% of ablation patients demonstrated complete eradication of IM, as compared to 2.3% in the control group ($P < 0.001$). There was less disease progression in patients in the ablation group (3.6% *vs* 16.3%, $P = 0.03$) and fewer cancers developed (1.2% *vs* 9.3%, $P = 0.045$). There were more reports of chest pain after ablation than after sham procedures, and a 6% esophageal stricture rate was reported in the treated group[40]. This stricture rate is markedly lower than that commonly reported for EMR, which confers a significant advantage for RFA in treatment of BE with flat HGD.

In patients who demonstrate visible lesions in the setting of HGD, a combination of EMR and RFA has recently been studied. Pouw and colleagues have reported on performance of EMR for visible lesions with subsequent ablation of the remaining segment[41]. Complete histological eradication of all dysplasia and IM was achieved in 43 patients (98%). Post-ablation complications included mucosal laceration at prior EMR sites (n = 3) and transient dysphagia (n = 4). No dysplasia recurred after a 21-mo follow-up period[41]. A more recent multicenter European trial involved EMR of visible lesions, followed by serial RFA. Focal escape endoscopic resection was utilized in cases of BE persistence despite RFA. The study

included 24 patients, and achieved neoplasia and IM eradication in 95% and 88% of patients, respectively. These rates improved to 100% and 96%, respectively, following escape EMR in two patients. No neoplasia recurred within a median 22-mo follow-up period[42]. Neo-squamous epithelium rigorous EMR and biopsy evaluation in a group of 22 post-RFA patients with baseline BE with IMC or HGD showed no evidence of persistent genetic abnormalities or buried BE glands[43]. To date, as far as we are aware, no published studies exist on outcomes of sole RFA therapy of BE with IMC.

CRYOTHERAPY

Cryotherapy is the latest modality to arrive on the endoscopic horizon of ablative options. This technology utilizes sprayed liquid nitrogen freeze-thaw cycles that result in tissue destruction by intracellular disruption and tissue ischemia, with relative preservation of the extracellular matrix to promote less fibrosis formation[44,45]. The procedure requires placement of an orogastric decompression tube to allow for adequate excess nitrogen gas expulsion to prevent inadvertent gastrointestinal viscus perforation. Repeat treatment sessions can be conducted every 4-6 wk as needed to ensure complete remission of the target area.

In a prospective open-label trial, Dumot *et al*[46] enrolled patients with BE and HGD or IMC who were not deemed surgical candidates or who refused esophagectomy. EMR was used for pathological staging of nodular areas before cryoablation and focal residual areas during the follow-up period. Patients with prior ablation therapy were not excluded. Twenty-seven of 30 patients had pathological downgrading post-treatment. After a median follow-up of 1 year, elimination of cancer or downgrading of HGD was achieved in 80% of IMC and 68% of HGD patients. A perforation occurred in a patient with Marfan syndrome, with the prototype system. Of six patients who showed a complete response, three had recurrence of dysplasia or cancer in the gastric cardia.

The efficacy and safety of liquid nitrogen cryotherapy has been demonstrated in a four-center study of 23 patients (17 with HGD, four with IMC, and three with early-stage adenocarcinoma). Complete response to HGD was found in 94% with HGD, and 100% with IMC and cancer. Complete response to IM was noted in 53% with HGD, 75% with IMC, and 67% with cancer. No symptoms were reported in 48% of 323 procedures. Esophageal strictures developed in three patients, but all were successfully treated by dilation. Other complications included chest pain, dysphagia, sore throat, and the gastric perforation noted in the Marfan patient as above[47].

CONCLUSION

BE early neoplasia treatment has undergone transition from radical esophagectomy to endoscopic organ-preserving options. The key to successful endoscopic management hinges on appropriate selection of candidate patients and detection of visible lesions through careful white light, high-definition endoscopy and ancillary imaging techniques such as narrow-band imaging and/or endomicroscopy. All visible lesions must be removed by EMR for definitive histopathological staging and to ensure adequacy of resection margins. Total eradication of the entire BE segment must occur to protect against synchronous/metachronous lesion development.

As a result of the higher risk of stricture development associated with EMR, our center currently employs a hybrid approach to treatment of BE early neoplasia that is based on segment length. For BE segments that measure $\leqslant$ 5 cm and harbor HGD or IMC, a CBE-EMR approach is used. For patients with BE segments > 5 cm, all focal lesions are resected, and the remaining flat BE is ablated using RFA to decrease the rate of stricture formation.

The critical research issues that still remain unanswered for endoscopic BE management center on: long-term survival and remission rates of both treated neoplasia and IM; development and significance of buried BE glands; quality of life and cost assessments for the various modalities compared to surgical cohorts; the role of these therapies for LGD or non-dysplastic BE; and the clinical impact of post-endoscopic therapy surveillance.

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TOPIC HIGHLIGHT

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Esophageal resection for high-grade dysplasia and intramucosal carcinoma: When and how?

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Abstract

High-grade dysplasia (HGD) and intramucosal carcinoma (IMC) in the setting of Barrett's esophagus have traditionally been treated with esophagectomy. However, with the advent of endoscopic mucosal resection and endoscopic ablative therapies, endoscopic therapy at centers with expertise is now an established treatment of Barrett's-esophagus-related neoplasia, including HGD and IMC. Esophagectomy is today reserved for more selected cases with submucosal invasion, evidence for lymph node metastasis, or unsuccessful endoscopic therapy.

Key words: Barrett's esophagus; High-grade dysplasia; Intramucosal carcinoma; Endoscopic mucosal resection; Esophagectomy

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INTRODUCTION

High-grade dysplasia (HGD) and intramucosal carcinoma (IMC) in the setting of Barrett's esophagus (BE) have traditionally been treated with esophagectomy. However, with the advent of endoscopic mucosal resection and endoscopic ablative therapies, endoscopic therapy at centers with expertise is now an established treatment of Barrett's-esophagus-related neoplasia, including HGD and IMC. Esophagectomy is today reserved for more selected cases with submucosal invasion, evidence for lymph node metastasis, or unsuccessful endoscopic therapy. This review highlights the updated role of and approaches for esophagectomy in the management of HGD and IMC in BE and discusses risk factors associated with submucosal invasion, lymph node metastasis, or unsuccessful endoscopic therapy.

TRADITIONAL APPROACH: ESOPHAGECTOMY AS THE STANDARD OF CARE FOR HGD

HGD in the setting of BE has been identified as a key risk factor in the progression to esophageal adenocarcinoma (EA). Patients with HGD are at a higher risk for progressing to EA than are patients with BE with no or low-grade dysplasia (LGD). This has given rise to performing prophylactic esophagectomy for the treatment of HGD to prevent EA. In addition to the risk of progression to EA, the surgical literature has reported a high risk of coexisting adenocarcinoma in patients with HGD that is

not diagnosed by endoscopic biopsy. The esophagectomy literature has reported varying prevalence of occult EA in patients with BE and HGD, ranging from 0% to 73%, and frequently approximates to a rate of around 40%[1-7]. Thus, the role of esophagectomy for the treatment of HGD is underlined by both prevention of cancer and cure of occult cancer.

Concerns have previously been raised as to whether esophagectomy is appropriate for most patients with HGD and IMC. Newer data have suggested that the incidence of invasive cancer is probably much lower than the 40% rate previously estimated[8]. This suggests that esophagectomy for HGD is unnecessary in more than 80% of patients in whom it is performed. At the same time, newer endoscopic techniques for evaluating and managing HGD and IMC have been developed and clinically tested. Currently, the approach to HGD and IMC is more complex and provides much more individualized care of patients than previously was available.

ENDOSCOPIC EVALUATION OF BARRETT'S-ESOPHAGUS-RELATED NEOPLASIA

The management of BE has been greatly influenced by the advent of endoscopic mucosal resection (EMR) and endoscopic ultrasound (EUS). Prior to the advent of endoscopic ablative techniques, whether intramucosal cancer was different from invasive cancer was a moot point, given that esophagectomy was indicated in either case. However, with endoscopic therapy now available for IMC, the distinction must be acknowledged. When evaluating treatment options it is crucial to understand the difference between the presence of intramucosal cancer limited to the mucosal lining, which only has a minimal nodal metastasis risk[9-11] and might be locally treatable, and the presence of cancer with invasion into the submucosa, which carries a higher nodal metastasis risk and requires surgery and/or systemic therapy[9,12-16].

Understanding pathological definitions is instrumental in managing a patient with Barrett's-related neoplasia. Dysplasia is neoplastic cytological and architectural atypia without evidence of invasion past the basement membrane. The diagnosis of LGD or HGD is based on the severity of cytological criteria that suggest neoplastic transformation of the columnar epithelium[17]. HGD and carcinoma *in situ* are regarded as equivalent. IMC is tumor that is limited to the lamina propria and is considered T1a by the American Joint Committee on Cancer staging. Submucosal carcinoma (SMC) is a tumor that invades past the muscularis mucosa into the submucosa, but not into the muscularis propria. Vessel invasion might be either venous or lymphatic channel invasion.

In a systematic review of the surgical literature that has reported the rates of cancer in patients who were undergoing esophagectomy for prophylactic treatment of HGD, the pooled average was 39.9% in the 441 patients who underwent esophagectomy for HGD among 23 studies[5]. These rates were largely based on retrospective studies with varying aims, sizes, definitions, and methodology. This average rate is consistent with previous pooled studies by Edwards *et al*[1], Ferguson *et al*[6], and Pellegrini *et al*[7] who have reported rates of 41%, 43% and 47%, respectively. However, the majority of these patients had IMC, whereas the rate of submucosal invasive cancer was decreased to 12.7% when applying both standardized criteria and strict definitions.

Prospective studies with rigorous endoscopic criteria in the EMR literature have reported lower rates of occult submucosal invasive disease. Among patients presenting with HGD and IMC who were undergoing complete BE EMR, the rate of occult submucosal invasive cancer was 4%[18]. Pech *et al*[19] have reported their long-term experience with EMR and other ablative procedures for Barrett's-esophagus-related neoplasia. They achieved a complete response in 96.6% and the 5-year survival rate was 84%. In their experience, esophagectomy was required in only 3.7% of patients initially presenting with HGD or IMC[19].

The management of HGD and IMC has now shifted from esophagectomy to endoscopic therapy to achieve total Barrett's eradication[18,20,21]. The concept of total Barrett's eradication highlights the importance of not only treating the known neoplasia, but also eradicating all of the at-risk Barrett's epithelium, to treat any synchronous lesions and hopefully prevent any metachronous lesions. Although expertise might vary from site to site and patient characteristics need to be taken into account, there is now acceptance of endoscopic therapy for HGD and IMC, and esophagectomy is no longer the standard of care[22].

Endoscopic modalities include tissue-acquiring therapies that include focal EMR, complete Barrett's EMR, and endoscopic submucosal dissection. Tissue-acquiring modalities are important to stage a visible lesion in the setting of HGD or for the treatment of IMC. HGD might also be treated with ablative therapies, such as photodynamic therapy, which has the longest experience of the ablative therapies[23], radiofrequency ablation, which has demonstrated initial success[24], and cryotherapy, which is a newer modality[25]. Chennat and Waxman have described these endoscopic therapies in further detail in their article in this issue.

HIGH-RISK CHARACTERISTICS OF BARRETT'S NEOPLASIA

Endoscopic therapy has advantages in that it is organ-preserving and does not have the same morbidity and mortality as surgery. However, not all cases are successful or appropriate for endoscopic therapy. Indications for esophagectomy include lymph node metastasis and failure of endoscopic therapy. Risk factors for submucosal invasion, lymph node metastasis, and failure of endoscopic treatment need to be incorporated into the management strategy of a patient with HGD and IMC. These risk factors are evident in endoscopic appearance, pathological characteristics, and results of endoscopic treatment (Table 1).

Table 1 High-risk characteristics associated with submucosal invasion, lymph node metastasis, or unsuccessful endoscopic therapy

Endoscopic characteristics
Long-segment Barrett's esophagus
Visible lesions with high risk endoscopic characteristics
Polypoid mass
Excavated lesions or ulcers
Evidence of lymph node involvement by EUS + FNA
Pathological characteristics
Multifocal HGD
Evidence of submucosal invasion
Deeper two thirds of the submucosa carries high risk of lymph node metastasis
Moderately or poorly differentiated tumor
Evidence of lymphatic channel invasion
Evidence of vascular invasion
Evidence of neural invasion
Treatment characteristics
Failure of ablation of remainder for Barrett's epithelium
Piecemeal endoscopic resection (as opposed to *en bloc* resection)
Longer time to achieve eradication

EUS: Endoscopic ultrasound; FNA: Fine needle aspiration; HGD: High-grade dysplasia.

Endoscopic characteristics

Long-segment BE has been identified as a risk factor for cancer[26] and for recurrence of neoplasia with endoscopic therapy[19]. Furthermore, visible lesions in the setting of HGD are more at risk for harboring occult cancer than flat dysplasia[5,27,28].

Careful white light examination is essential for targeting biopsies and resection of visible lesions because visible lesions in the setting of dysplasia have a high risk of occult cancer. Furthermore, the type of lesion is correlated with risk of submucosal invasion. Standardization of endoscopic appearance of visible lesions is now developing, and more attention is being given to non-protruding lesions. The updated Paris classification is based on the Japanese classification of gastric lesions. In the esophagus, superficial lesions based on endoscopic appearance include the following classifications: protruding pedunculated (type 0- Ⅰ p), protruding sessile (0- Ⅰ s), slightly elevated (0- Ⅱ a), completely flat (0- Ⅱ b), slightly depressed (0- Ⅱ c), excavated (0-Ⅲ), or a mixed pattern[29]. Type 0-Ⅲ is suspicious for submucosal invasion. Type 0- Ⅰ and type 0- Ⅱ c lesions are also associated with increased risk of submucosal penetration[30]. Thus, protruding or depressed lesions are at higher risk than those slightly raised or flat areas. EMR provides an opportunity to stage the depth of a lesion in areas of question.

Endoscopic ultrasound in BE demonstrates a thickened mucosal lining. It is not optimal for differentiation between a T1a tumor (IMC) and a T1b (SMC) tumor, and EMR is better suited for depth staging at this range[31]. However, given the risk of lymph node metastasis in patients with IMC, EUS with fine needle aspiration (FNA) might identify patients not eligible for endoscopic therapy[32]. EUS with or without FNA is a reasonable procedure in all patients with IMC and patients with visible lesions, who have a higher risk of occult cancer. Any patient found with lymph node involvement should be referred for esophagectomy. The utility of EUS in flat HGD might be questioned[33].

Pathological characteristics

The diagnosis of HGD, IMC, and invasive cancer represents a biological and histological continuum. Although pathological assessment is the gold standard, interpretation is subject to a great deal of variability among pathologists. There is high inter-interpreter variability in diagnosing HGD as reported in the literature[34-38]. Due to limited sample size and depth, as well as potential crush artifacts, pathologists might not reliably be able to distinguish between HGD, IMC and SMC on a single biopsy specimen. One of the advantages of EMR specimens is that pathologists are better able to stage lesions because they provide large and intact pathological specimens.

In evaluations of specimens from EMR for Barrett's neoplasia, moderately or poorly differentiated cancers are more likely to invade the submucosa[30,39]. HGD obtained from multiple levels throughout a BE segment has a higher risk of being associated with occult cancer[28]. Furthermore, in a risk analysis performed on patients with either HGD or IMC, multifocal neoplasia has been cited as a risk factor for recurrence after endoscopic therapy[19]. Risk factors for lymph node metastasis in EA are vascular invasion, lymphatic channel permutation, neural invasion, and grade of the tumor[40,41]. In EA, submucosal invasion of the most superficial third does not carry the same lymph node metastasis risk as the deeper two thirds[40]. Manner *et al*[42] have reported favorable outcomes with endoscopic resection of low-risk SMC in their long-term experience of endoscopic resection. However, larger trials are needed before adopting endoscopic therapy as standard practice for these superficial submucosal invading tumors.

Treatment characteristics

Endoscopic resection specimens not only provide a histological specimen that is important for accurate pathological diagnosis, but also provide a means for assessing treatment adequacy. Lateral margins might indicate that further endoscopic treatment is necessary, whereas positive deep margins indicate that surgery is appropriate. The following are associated with a higher risk of recurrence: length of time to complete eradication of neoplasia with multiple endoscopic treatment sessions; piecemeal resection; and no ablative therapy to target the remainder of the at-risk Barrett's epithelium[19].

Although there is ongoing interest and early investigations for genetic or molecular markers to predict endoscopic response[43], none of these markers has been validated for clinical use.

ADVANTAGES OF ESOPHAGECTOMY

The strategy of performing esophagectomy for HGD or IMC not only cures the index condition, but also addresses occult cancer and prevents cancer death[44]. Although endoscopic treatment is an appropriate and cost-effec-

tive[45] approach for the treatment of many patients with HGD and IMC, patients who are appropriate surgical candidates can benefit from esophagectomy. The surgical specimen enables accurate staging of disease to diagnose areas of occult cancer, and confirms treatment adequacy with negative margins and lymph nodes. Conventional approaches are transhiatal esophagectomy and transthoracic esophagectomy. Minimally invasive esophagectomy (MIE) techniques are growing in popularity because of their perceived benefits of reduced pain, lower incidence of postoperative complications, and faster recovery. These MIE techniques include video-assisted thoracoscopy surgery with laparotomy or laparoscopy, laparoscopy with a right thoracotomy, or laparoscopic transhiatal resections. These procedures have been studied in mostly retrospective studies and conclusions are limited in terms of direct comparisons to open surgery due to lack of prospective randomized trials[46,47].

The issue of the morbidity and mortality of esophagectomy is the major concern for either open esophagectomy or MIE. Adverse outcomes include pulmonary complications, hemorrhage, anastomotic leakage, infections, and recurrent nerve palsy. Although one study based on a national Veteran's Affairs database has reported morbidity of almost 50% and mortality of 10%[48], the expertise and volume of the center, the experience of the surgeon, the patient risk factors, and the indications for esophagectomy should be taken into account[49-51]. In institutions with expertise and high volumes, the mortality rate is 2%-3%[52]. It is also important to note that esophagectomy specifically for HGD has a different risk profile than that of esophagectomy for cancer. Comorbid diseases, debilitation from cancer and/or neoadjuvant therapy, and issues with locally advanced disease are not as predominant in patients with HGD. A pooled mortality rate of 1% was calculated among six studies that involved esophagectomy for HGD[49]. Quality of life indicators for patients who underwent esophagectomy for HGD and IMC are equivalent to those of the general population[53].

INDICATIONS FOR ESOPHAGECTOMY FOR BARRETT'S HGD OR IMC

Strong indications for esophagectomy include lymph node metastasis and failure of endoscopic therapy. Invasion of tumor into the submucosa is still considered a strong indication for esophagectomy, although invasion into the superficial third of the submucosa does not carry the same lymph node metastasis risk as the deeper two thirds, and potentially could be treated endoscopically[29,42]. Factors to consider in the management strategy for HGD and IMC include characteristics that are associated with lymph node metastasis, submucosal invasion, and failure of endoscopic therapy, as listed in Table 1, and may serve as milder indications for esophagectomy. Excavated lesions (Paris classification 0-Ⅲ) are not typically considered to be amenable to endoscopic therapy due to high suspicion of submucosal invasion, whereas protruding lesions (0-Ⅰ) and depressed lesions (0-Ⅱc) are a concern for submucosal invasion and should be approached with caution endoscopically (Table 2). These circumstances allow for endoscopic resection to serve as a diagnostic tool to stage the lesion accurately to determine if the lesion is amenable to endoscopic therapy. Multifocal high grade is a milder indication for esophagectomy than previously considered, due to the evolving options of ablative therapy. These risk factors, as listed in Table 1, need to be weighed with patient characteristics, patient preferences, available surgical expertise, available endoscopic expertise, and surgical approach options to decide if esophagectomy or endoscopic therapy is appropriate for each case.

Table 2 Relative risk of submucosal invasion associated with endoscopic appearance of lesions

Endoscopic appearance	Paris classification	Relative risk of submucosal invasion
Polypoid	0-Ⅰp	Higher
Sessile	0-Ⅰs	Higher
Slightly raised	0-Ⅰa	Low
Flat	0-Ⅰb	Low
Slightly depressed	0-Ⅰc	Higher
Excavated	0-Ⅲ	Very high

WHICH OPERATION FOR BARRETT'S HGD OR IMC?

Selection of the appropriate approach to esophagectomy for HGD or IMC is based on a number of factors (Table 3). Prior surgery in the chest or abdomen might require an open rather than a minimally invasive approach, and prior esophageal surgery such as fundoplication might limit consideration of a vagal-sparing approach. Comorbidity such as severe pulmonary disease, or advanced age might encourage some surgeons to pursue an approach associated with less postoperative pulmonary morbidity, such as transhiatal esophagectomy[54]. Whether minimally invasive approaches offer a lower risk of postoperative pulmonary morbidity compared to open transthoracic approaches has not yet been adequately determined[47,55-57].

The appropriate extent of operation for HGD or IMC is somewhat complex and controversial, and is related to the length of esophagus that must be resected, the extent of soft tissue resection around the esophagus, and the regions for lymph node dissection. It is appropriate to examine the surgical specimen at the time of resection, and usually to perform a frozen section analysis of the proximal margin, to ensure that all the Barrett's mucosa has been removed. Limiting the resection to encompass just the Barrett's segment is probably not a good long-term strategy, because most reconstructive techniques using a gastric tube create a model of frequent reflux, thus exposing patients to the possibility of developing Barrett's mucosa in the remaining esophagus[58]. Indeed, this phenomenon has been well documented in the esophageal remnant after standard subtotal esophagectomy, and theoretically, the risk would be increased if more esophagus were left in place[58-64]. Some cases of adenocarci-

Table 3 Selecting an appropriate surgical approach

Patient characteristics
Prior surgery (thoracic, abdominal, esophageal)
Obesity
Age
Pulmonary function
Other comorbid factors
Surgical options
Standard open resection
Transhiatal esophagectomy (2 or 3 holes)
Minimally invasive esophagectomy
Vagus sparing esophagectomy
Mucosal stripping esophagectomy?
Extent of operation
Extent of esophageal resection
Limited resection of Barrett's segment
Near-total esophagectomy
Extent of soft tissue resection
Minimal
Standard
Extended
Extent of nodal dissection
Minimal
Standard
Extended 3-field
Surgical results
Accuracy of staging
Number of lymph nodes
Effects on long-term survival
Effects on perioperative outcomes

noma arising in such metaplastic epithelium have been described[65,66]. Therefore, a near total esophagectomy is recommended for patients who are undergoing esophagectomy for HGD or IMC.

The lateral extent of soft tissue resection for HGD or IMC is a more controversial problem, with the possible range extending from a vagal-sparing esophagectomy, in which no additional soft tissues are removed, to an extended *en bloc* esophagectomy, which sometimes includes the azygos vein, thoracic duct, contralateral pleura, a rim of diaphragm, and in some cases, even the posterior pericardium. With the increasing accuracy of EUS in assessing the depth of penetration of the primary tumor, anything more than removing a standard amount of soft tissue representing the lateral margins is not likely to provide the patient with benefits regarding local recurrence, but might add to postoperative morbidity. Whether a vagal-sparing operation offers the same freedom from local recurrence has not been sufficiently studied to date[67].

The appropriate extent of nodal dissection for HGD or IMC is also controversial. In order to stage esophageal cancer accurately it has been suggested that a minimum of 10 lymph nodes be resected for early-stage cancers[68]. The use of more extensive nodal dissections, especially three-field lymphadenectomy, are controversial for regionally advanced cancers and are likely inappropriate for HGD and IMC, although this question has not been formally studied.

The best surgical option for HGD or IMC is the one that produces the least morbidity, balanced against the best long-term survival. As present, any standard resection technique including open transthoracic, minimally invasive, and transhiatal approaches provide similar long-term outcomes, and transhiatal esophagectomy might have an advantage in reducing postoperative morbidity. The more extensive resections (open transthoracic, and minimally invasive) are likely to improve staging accuracy, particularly with regards to nodal status. Long-term functional status is similar regardless of the surgical approach. The use of vagal-sparing techniques, especially for HGD, has potentially interesting advantages with regard to quality of life, but has not been adequately evaluated in terms of staging accuracy and long-term outcomes. In the end, it is the surgeon's training and experience, in combination with the individual patient's needs that determines the most appropriate approach to esophagectomy for HGD or IMC.

CONCLUSION

Barrett's HGD or IMC can be primarily treated endoscopically with endoscopic resection and endoscopic ablation with the goal of total Barrett's eradication. Evidence of submucosal invasion, lymph node metastasis or failure of endoscopic therapy or their risk factors, which can be ascertained by endoscopic appearance, pathological characteristics, and treatment course, need to be incorporated into the decision-making process for endoscopic versus surgical treatment. Longer-term studies with additional risk analysis need to be carried out to be able to predict reliably which patients are amendable to endoscopic therapy and who may benefit from esophagectomy.

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TOPIC HIGHLIGHT

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Neoadjuvant treatment of esophageal cancer

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Abstract

The management of esophageal cancer has been evolving over the past 30 years. In the United States, multimodality treatment combining chemotherapy and radiotherapy (RT) prior to surgical resection has come to be accepted by many as the standard of care, although debate about its overall effect on survival still exists, and rightfully so. Despite recent improvements in detection and treatment, the overall survival of patients with esophageal cancer remains lower than most solid tumors, which highlights why further advances are so desperately needed. The aim of this article is to provide a complete review of the history of esophageal cancer treatment with the addition of chemotherapy, RT, and more recently, targeted agents to the surgical management of resectable disease.

Key words: Esophageal cancer; Multimodality therapy; Neoadjuvant therapy; Chemotherapy; Radiotherapy; Targeted agents; Disease management

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INTRODUCTION

Esophageal cancer is the most rapidly increasing tumor type in the Western world[1,2]. Globally, esophageal cancer is the eighth most common malignancy and sixth most fatal, with approximately 460 000 new diagnoses and > 380 000 deaths annually[3]. The lifetime risk, as well as histology of esophageal cancer varies worldwide from 1 in 200 in the United States, with more than half of new cases being adenocarcinoma (AC) to more than 10 times that risk in Iran, Northern China, India, and Southern Africa, where the histology is > 90% squamous cell carcinoma (SCC), and mirrors the growing epidemic of tobacco abuse[3-5].

Although there are multiple, rare esophageal cancer histologies (e.g. gastrointestinal stromal tumors, leiomyosarcoma, and liposarcoma), AC and SCC are the two principle variants and account for > 98% of esophageal cancer diagnoses[6]. Historically, AC and SCC have been treated as a single disease entity with many older clinical trials not differentiating between the two histologies, even in study populations[7]. Over the years, however, a great deal of evidence has been compiled to support the notion that AC and SCC represent two separate diseases based on their differing etiology, epidemiology, prognosis, and response to treatment[8-11].

AC is highly associated with obesity and gastroesophageal reflux disease (GERD). Obesity increases the risk of developing GERD by approximately twofold due to elevated intra-abdominal pressure and a resultant laxity in the lower esophageal sphincter[12]. GERD leads to chronic

irritation of the distal esophagus and can eventually cause metaplasia by the replacement of normal, squamous epithelium by columnar epithelium and the formation of what is referred to as Barrett's esophagus. The new, secretory columnar cells are thought to be better-suited to withstand the erosive contents that spill over from the gastroesophageal junction (GEJ), but unfortunately, this change also increases the risk for dysplasia by sevenfold, with Barrett's esophagus evolving to AC at a rate of approximately 1% per year[13,14].

SCC, on the other hand, is almost always linked to tobacco and alcohol abuse. Current smokers have a ninefold increased risk of developing SCC of the esophagus, while heavy drinkers of alcohol have an increased OR of 5[15]. Combined, however, the synergistic effects of tobacco and alcohol abuse lead to a 20-fold increased risk of developing esophageal cancer[16], although more extreme abusers of the two have been reported to have an increased OR as high as 50 and even 107 in studies from Italy and South America, respectively[17,18].

Epidemiologically, there has been a dramatic shift in the two histologies[5]. In the United States between 1974 and 1994, there has been a staggering 350% increase in the number of patients with esophageal AC, which now represents 60% of all new esophageal cancer diagnoses. Prior to 1974, SCC constituted 90% of esophageal cancer in the United States, which was likely secondary to increased rates of tobacco abuse[5,19]. The median age of diagnosis for SCC is approximately one decade prior to that of AC, yet surprisingly, patients with SCC have been documented in more recent studies to fair worse[7-9,20,21]. This difference is likely to be secondary to the increased comorbidity of patients with SCC but, even more importantly, the location of the primary tumor. Compared to age and lung function, the adjusted OR for postoperative death for a tumor located in the upper third of the esophagus is 4[7,22]. SCC is usually a proximal lesion, with 75% of these cancers found to have contact with the tracheobronchial tree, while 94% of ACs are below the tracheal bifurcation[7].

With regard to location, it should be noted that the pathology, treatment and prognosis of SCC of the cervical esophagus are more closely related to that of SCC of the head and neck[23]. As such, this review instead focuses on the multimodality treatment of localized and locoregional cancer involving the thoracic esophagus and GEJ. The definition of what constitutes the GEJ is debatable in itself. Siewert and Stein have described the most accepted classification scheme for AC at the GEJ: type Ⅰ, AC arising from an area of intestinal metaplasia of the esophagus, which can infiltrate the GEJ from above; type Ⅱ, AC arising from the cardia of the stomach; type Ⅲ, subcardial gastric carcinoma that infiltrates the GEJ from below[24]. With the exception of overexpression of *COX-2* with type I GEJ AC, no known significant gene expression profile changes have been noted that differentiate the three sub-types consistently[25]. Type I GEJ tumors tend to have lymphatic drainage toward lower mediastinal and upper gastric lymph nodes, whereas type Ⅱ and Ⅲ GEJ tumors are more likely to drain to celiac axis nodes. As such, type Ⅰ GEJ tumors are generally treated as distal esophageal cancer, whereas type Ⅱ and Ⅲ GEJ tumors are viewed by many as gastric carcinomas[24,25].

TREATMENT

Surgery alone

Debate regarding the current standard of care for the management of esophageal cancer is ongoing[26-28]. Surgical resection alone has been the mainstay of treatment for decades[29], although its necessity has been called into question more recently for patients with SCC[30,31]. Although surgery is considered to offer the best chance of prolonged survival, alone it will only cure 15%-20% of patients with localized disease[32-35], and unfortunately, 50%-60% of patients with esophageal cancer have tumors that are considered inoperable, secondary to either tumor extension or medical comorbidity[29]. Contemporary outcome data for treatment with surgery alone report a median survival of 16 mo with a 1-, 2- and 3-year survival rate of 60%, 37% and 26%, respectively[32]. Local disease-failure rates with surgery alone are quite high at 58%, with two-thirds of those failures from lack of complete (R0) resection and one-third recurring locally despite an R0 resection[36]. Surgical approaches and techniques - transthoracic *vs* transhiatal resection with limited *vs* extended-field lymphadenectomy - are highly debated[34,35], and are beyond the scope of this review. What is clear, however, is that postoperative morbidity and mortality are decreased while overall survival (OS) is significantly improved in high-volume, expert academic centers[37,38]. Currently, National Comprehensive Cancer Network guidelines suggest surgery as a single-modality treatment option only for non-cervical T1 lesions without lymph node involvement[39].

Radiotherapy

Radiotherapy alone has been the historical treatment of choice for patients with esophageal cancer who are not surgical candidates. Radiotherapy delivered at 60-66 Gy over 6-6.5 wk has been associated with a 5-year OS ranging from 5% to 20% depending on tumor extent[40-42]. In a review by Earlam and colleagues, 49 earlier series that involved 8489 patients with SCC treated with radiotherapy alone have been reported to yield a 1-, 2- and 5-year survival rate of 18%, 8% and 6%, respectively[43]. Adding radiotherapy to the surgical management of esophageal cancer has the advantage of increasing local control of disease. In the adjuvant setting, radiotherapy can treat microscopic disease left behind after an incomplete surgery. In the neoadjuvant setting, radiotherapy can theoretically decrease the size of a lesion prior to surgery and potentially make that lesion more resectable. The obvious trade-off of increased local control with radiotherapy is poor wound healing in both settings and an increasingly difficult resection of previously irradiated tissue in the neoadjuvant setting.

As it stands, there have been five separate phase Ⅲ trials that have compared adjuvant radiotherapy with sur-

Table 1 Randomized controlled trials of adjuvant radiotherapy *vs* surgery alone for esophageal cancer

Studies	Histology	Treatment	*n*	MS (mo)	5-yr OS (%)	*P*	RT dose (Gy)
Kunath *et al*[44], 1984	SCC	ART	23	9		NS	50-55
		Surgery	21	6			
Ténière *et al*[45], 1991	SCC	ART	102	18	19	NS	45-55
		Surgery	119	18	19		
Fok *et al*[36], 1993	SCC	ART	42	11	10	NS	43-53
		Surgery	39	22	16		
Zieren *et al*[46], 1995	SCC	ART	33		23[1]	NS	56
		Surgery	35		22[1]		
Xiao *et al*[47], 2003	AC/SCC	ART	220		41	NS	50-60
		Surgery	275		32		

[1]3-year OS. MS: Median survival; RT: Radiotherapy; SCC: Squamous cell carcinoma; AC: Adenocarcinoma; ART: Adjuvant radiotherapy; NS: Not significant; OS: Overall survival.

Table 2 Randomized controlled trials of neoadjuvant radiotherapy *vs* surgery alone for esophageal cancer

Studies	Histology	Treatment	*n*	MS (mo)	5-yr OS (%)	*P*	RT dose (Gy)
Launois *et al*[48], 1981	SCC	NART	77	10	10	NS	40
		Surgery	57	12	12		
Gignoux *et al*[49], 1987	SCC	NART	106	11	11	NS	33
		Surgery	102	11	10		
Arnott *et al*[50], 1992	AC/SCC	NART	90	8	9	NS	20
		Surgery	86	8	17		
Nygaard *et al*[51], 1992	SCC	NART	48[1]		21[3]	NS	35
		Surgery	41[2]		9[3]		
Wang *et al*[52], 1989	SCC	NART	104		35	NS	40
		Surgery	102		30		

[1]Group 3: NART; [2]Group 1: Surgery alone; [3]3-year OS. MS: Median survival; RT: Radiotherapy; SCC: Squamous cell carcinoma; AC: Adenocarcinoma; NART: Neoadjuvant radiotherapy; NS: Not significant; OS: Overall survival.

gery alone[36,44-47] (Table 1), and another five phase Ⅲ trials that have compared neoadjuvant radiotherapy to surgery alone[48-52] (Table 2). Although local control of disease was improved in each of the adjuvant radiation arms, there were increased complications secondary to adhesions, scarring and fistulas, and none reported an OS advantage in their entire study population as a whole. Among these trials, however, Xiao and colleagues randomized 495 patients with SCC to surgery followed by adjuvant radiotherapy or to surgery alone. Although the 5-year OS was not statistically different for all-comers (41% *vs* 32%, $P = 0.45$), a 5-year OS advantage was noted in a subgroup analysis of patients with stage Ⅲ disease (35% *vs* 13%, $P < 0.003$), which favored the arm that received adjuvant radiotherapy[47].

Of the five phase Ⅲ trials that have evaluated neoadjuvant radiotherapy in esophageal cancer, none has demonstrated an increase in resectability or OS in those treated with preoperative radiotherapy alone[48-52]. Although Nygaard and colleagues have reported a 3-year OS benefit, this was only after pooling patients who had received neoadjuvant radiotherapy with those who had also received neoadjuvant chemoradiotherapy, as there was no significant difference in survival found otherwise[51]. A meta-analysis of trials that have used neoadjuvant radiotherapy with a median follow-up of 9 years, and including data from 1147 patients who almost exclusively had SCC, has revealed a trend toward improved 5-year OS (OR: 0.89, 95% CI: 0.78-1.01, $P = 0.062$), but ultimately has failed to show a statistically significant survival advantage[53].

Chemotherapy

The theoretical advantages of adding chemotherapy to the treatment of esophageal cancer are for potential tumor down-staging prior to surgery, as well as targeting micrometastatic disease, and thus decreasing the risk of distant spread. Adjuvant chemotherapy with cisplatin-based regimens compared to surgery alone has been examined in three separate phase Ⅲ trials[54-56] (Table 3), with none of them reporting a statistically significant difference in OS, although Ando and colleagues have reported a 5-year disease-free survival (DFS) advantage (55% *vs* 45%, $P = 0.037$)[56]. In the neoadjuvant setting, there have been multiple randomized trials that have compared varying chemotherapeutic regimens to surgery alone[32,51,57-63] (Table 4). Clinical complete responses based on direct visualization and an assortment of imaging modalities have ranged from 19% to 58%, but the rate of pathological complete response (pCR) at the time of surgery was a disappointing 2.5%-13%. This is an unsurprising trend considering the relative ineffectiveness of chemotherapy alone in the treatment of esophageal cancer[32,51,57-63].

The UK Medical Research Council (MRC) trial included 802 patients of all histologies, and randomized patients

Table 3 Randomized controlled trials of adjuvant chemotherapy *vs* surgery alone for esophageal cancer

Studies	Histology	Treatment	*n*	MS (mo)	5-yr OS (%)	*P*
Pouliquen *et al*[54], 1996	SCC	CF	52	13		NS
		Surgery	68	14		
Ando *et al*[55], 1997	SCC	CV	100		45	NS
		Surgery	105		48	
Ando *et al*[56], 2003	SCC	CF	120		61	NS
		Surgery	122		52	

MS: Median survival; SCC: Squamous cell carcinoma; C: Cisplatin; F: Fluorouracil; V: Vindesine; NS: Not significant; OS: Overall survival.

Table 4 Randomized controlled trials of neoadjuvant chemotherapy *vs* surgery alone for esophageal cancer

Studies	Histology	Treatment	*n*	MS (mo)	3-yr OS (%)	*P*
Schlag *et al*[57], 1992	SCC	CF	22	7		NS
		Surgery	24	6		
Nygaard *et al*[51], 1992	SCC	BC	44	7	3	NS
		Surgery	41	7	9	
Maipang *et al*[58], 1994	SCC	BVC	24	17	31	NS
		Surgery	22	17	36	
Law *et al*[59], 1997	SCC	CF	74	17	40	NS
		Surgery	73	13	13	
Kelsen *et al*[32], 1998	AC/SCC	CF	213	15	19[1]	NS
		Surgery	227	16	20[1]	
Ancona *et al*[60], 2001	SCC	CF	47	25	34[1]	NS
		Surgery	47	24	22[1]	
MRC[61], 2002	AC/SCC	CF	400	17	43	< 0.01
		Surgery	402	13	34	

[1]5-year OS. MS: Median survival; SCC: Squamous cell carcinoma; AC: Adenocarcinoma; C: Cisplatin; F: Fluorouracil; B: Bleomycin; V: Vindesine; NS: Not significant; OS: Overall survival.

to two cycles of neoadjuvant cisplatin 80 mg/m^2 and infusional fluorouracil 1000 mg/m^2 per d for 4 d *vs* surgery alone. A rather striking distinction of this trial compared to others was that clinicians could give their patients neoadjuvant radiotherapy (25-32.5 Gy) irrespective of randomization, and 9% of patients on each arm received radiotherapy. R0 resections were reported in 60% of assessable patients that were treated with neoadjuvant chemotherapy *vs* 54% of patients treated with surgery alone ($P < 0.0001$). OS was also improved in the neoadjuvant group (HR: 0.79, 95% CI: 0.67-0.93, $P = 0.004$), with a median OS of 16.8 mo *vs* 13.3 mo, respectively[61]. Another large trial by Kelsen *et al*[32] has evaluated neoadjuvant chemotherapy in the Intergroup (INT) 0113 study with 440 patients, however, reported no difference in OS was reported. Two large meta-analyses also have failed to demonstrate a survival advantage with neoadjuvant chemotherapy[64,65], although another meta-analysis by Gebski *et al*[66] has reported a statistically significant OS benefit with neoadjuvant chemotherapy (HR: 0.90, 95% CI: 0.81-1.00, $P = 0.05$), which corresponds to a 2-year absolute survival benefit of 7%. Caveats to this meta-analysis are that no statistically significant benefit was seen for patients with SCC treated with neoadjuvant chemotherapy (HR: 0.88, 95% CI: 0.75-1.03, $P = 0.12$) and that, although there was a benefit seen with AC (HR: 0.78, 95% CI: 0.64-0.95, $P = 0.014$), these results were based solely on the single trial whose data were available for review - the MRC trial[61,66].

At least four separate trials have compared cisplatin-based perioperative regimens (neoadjuvant and adjuvant chemotherapy) to surgery alone in esophageal cancer[32,67-69] (Table 5). Those that focused solely on esophageal cancer did not reveal survival benefits[32,67], whereas the two that included patients with AC of the stomach and GEJ did show such a benefit[68,69]. The largest of these, published by Cunningham and colleagues, randomized 503 patients with AC to three preoperative and three postoperative courses of epirubicin 50 mg/m^2 and cisplatin 60 mg/m^2 with infusional fluorouracil 200 mg/m^2 per day for 21 d *vs* surgery alone. Although the majority of patients had gastric AC, approximately 26% of the patients enrolled had AC of the GEJ or distal esophagus. Despite the fact that 58% of patients were unable to tolerate all six cycles of chemotherapy, the perioperative chemotherapy group had a statistically significant higher likelihood of OS compared to those treated with surgery alone (HR: 0.75, 95% CI: 0.60-0.93, $P = 0.009$), with an improved median OS (24 mo *vs* 20 mo) and 5-year OS (36% *vs* 23%). Although postoperative complications were not increased (46% *vs* 45%), there was also no difference in the rate of R0 resection (69% *vs* 66%) or pCR (both 0%). Importantly, there was no evidence of heterogeneity of treatment effect based on the location of the primary tumor[68].

Chemoradiotherapy

Chemotherapy in conjunction with radiotherapy was

Table 5 Randomized controlled trials of perioperative chemotherapy *vs* surgery alone for esophageal cancer

Studies	Histology	Treatment	*n*	MS (mo)	5-yr OS (%)	*P*
Roth *et al*[67], 1988	AC/SCC	BVC	19	9	25	NS
		Surgery	20	9	5	
Kelsen *et al*[32], 1998	AC/SCC	CF	213[1]	15	19	NS
		Surgery	227	16	20	
Cunningham *et al*[68], 2006	AC[2]	ECF	250	24	36	NS
		Surgery	253	20	23	
Boige *et al*[69], 2007	AC[3]	CF	113[4]		38	< 0.05
		Surgery	111		24	

[1]Of 213 patients in the perioperative arm, only 66 later received adjuvant chemotherapy; [2]26% had AC of the GEJ and lower esophagus; [3]11% had esophageal AC; [4]Of 113 patients in the perioperative arm, only 54 later received adjuvant chemotherapy. MS: Median survival; SCC: Squamous cell carcinoma; AC: Adenocarcinoma; B: Bleomycin; C: Cisplatin; V: Vindesine; F: Fluorouracil; E: Epirubicin; NS: Not significant; OS: Overall survival.

initially evaluated as a definitive treatment for patients deemed unable to proceed with surgery[70]. In combination, chemotherapy not only compliments but augments the effect of radiotherapy in a process known as radiation sensitization, secondary to synergistic DNA damage, cell cycle synchronization, and inhibition of repair and resistance pathways[71,72]. In addition to increasing the efficacy of radiotherapy and thus controlling local tumor growth, as mentioned earlier, chemotherapy theoretically also offers the ability to eradicate micrometastatic disease and decrease the risk of distant recurrence[73].

The seminal Radiation Therapy Oncology Group (RTOG) 85-01 trial has compared radiotherapy (50.4 Gy over 5 wk) with concurrent cisplatin 75 mg/m^2 and infusional fluorouracil 1000 mg/m^2 per day for 4 d to radiotherapy alone (64 Gy over 6.4 wk). The chemotherapy arm consisted of four cycles delivered every 4 wk during radiotherapy (cycles 1 and 2) and every 3 wk for the remainder (cycles 3 and 4). The study included 134 patients with 90% having SCC and all with T1-3 N0-1 M0 disease. The trial was closed early once an interim analysis revealed that there was a statistically significant survival advantage that favored concurrent chemoradiotherapy that later amounted to a 5-year OS of 27% *vs* 0%. There was no statistically significant difference in OS based on histology[70].

Although those who received concurrent chemoradiotherapy had a decreased risk of persistent disease or local recurrence compared to those who received radiotherapy alone in the RTOG 85-01 trial, the incidence of locoregional failure was still 47%[70], and the INT 0123 trial was launched in an effort to improve upon this, with the theory that higher doses of radiotherapy would be beneficial. A total of 236 patients with T1-3 N0-1 M0 disease were enrolled (85% with SCC) and randomized to high-dose radiotherapy (64.8 Gy) *vs* low-dose radiotherapy (50.4 Gy), with both arms receiving four cycles of concurrent chemotherapy (cisplatin 75 mg/m^2 and infusional fluorouracil 1000 mg/m^2 per day for 4 d every 4 wk). The INT 0123 trial was also stopped early after an interim analysis failed to reveal a significant difference in median OS (13 mo *vs* 18.1 mo), 2-year survival (31% *vs* 40%), or locoregional persistence/recurrence of disease (56% *vs* 52%) between the high-dose and low-dose radiotherapy arms, respectively[74]. With such unacceptably high locoregional failure rates with definitive chemoradiotherapy, in addition to the dismal prognosis of patients treated with surgical resection alone[32-35], numerous trials were begun to evaluate multimodality treatments that combine chemotherapy, radiotherapy, and surgical resection.

To date, at least nine randomized phase Ⅲ clinical trials have compared neoadjuvant chemoradiotherapy with surgery alone[33,51,75-82] (Table 6). These trials incorporated multiple chemotherapy regimens, doses of radiotherapy used (20-50.4 Gy), and timing of radiotherapy with regard to chemotherapy (sequential *vs* concurrent), in addition to differing by surgical procedures performed and histological types of esophageal cancer enrolled (AC, SCC, or both). Only two of these trials have revealed a significant survival benefit that favored multimodality treatment, and neither was without its imperfections[77,81]. Walsh and colleagues randomized 113 patients with AC to two courses of neoadjuvant cisplatin 75 mg/m^2 and fluorouracil 15 mg/kg per day for 5 d with concurrent radiotherapy (40 Gy over 3 wk) or to surgery alone. The median OS was 16 mo *vs* 11 mo (P = 0.01) with a 3-year OS of 32% *vs* 6% (P = 0.01), which favored the multimodality treatment arm[77]. This single-institution-based trial, however, has been heavily criticized for an OS of patients with localized esophageal cancer treated with surgery alone (6%) that was far inferior to historical controls[32].

The second study, the Cancer and Leukemia Group B 9781 trial, was closed early with only 56 of an expected 500 patients enrolled, secondary to poor accrual that was reportedly due to the unwillingness of many patients and physicians to enroll in the control surgery-alone arm. Patients were randomly assigned to two cycles of cisplatin 100 mg/m^2 and fluorouracil 1000 mg/m^2 per day for 4 d with concurrent radiotherapy (50.4 Gy over 5.5 wk) prior to surgery, or to surgery alone. An impressive 5-year OS of 39% *vs* 16% was reported with a median OS of 4.48 years *vs* 1.79 years (P = 0.002), respectively. Although the obvious clinical significance of these findings is hard to dispute, a trial with more robust participation would have gone a long way to alleviate any uncertainties regarding the best treatment strategy for resectable esophageal cancer[81].

Table 6 Randomized controlled trials of neoadjuvant and adjuvant chemoradiotherapy *vs* surgery alone for esophageal cancer

Studies (yr)	Histology	Treatment	*n*	MS (mo)	5-yr OS (%)	*P*
Nygaard *et al*[51], 1992[1]	SCC	BC + 35 Gy	47	8	17[3]	NS
		Surgery	41	7	9[3]	
Apinop *et al*[75], 1994[1]	SCC	CF + 20 Gy	35	10	24	NS
		Surgery	34	7	10	
Le Prise *et al*[76], 1994[1]	SCC	CF + 20 Gy	41	10	19[3]	NS
		Surgery	45	11	14[3]	
Walsh *et al*[77], 1996[1]	AC	CF + 40 Gy	58	16	32[3]	< 0.05
		Surgery	55	11	6[3]	
Bosset *et al*[33], 1997[1]	SCC	C + 37 Gy	143	19	7	NS
		Surgery	139	19	9	
Urba *et al*[78], 2001[1]	AC/SCC	CFV + 45 Gy	50	17	20	NS
		Surgery	50	18	10	
Lee *et al*[79], 2004[1]	SCC	CF + 45 Gy	51	28	49[3]	NS
		Surgery	50	27	41[3]	
Burmeister *et al*[80], 2005[1]	AC/SCC	CF + 35 Gy	128	22	17	NS
		Surgery	128	19	13	
Tepper *et al*[81], 2008[1]	AC/SCC	CF + 50.4 Gy	30	54	39	< 0.01
		Surgery	26	21	16	
Macdonald *et al*[82], 2001[2]	AC[4]	F + 45 Gy	281	36	50[3]	< 0.01
		Surgery	275	27	41[3]	

[1]Neoadjuvant chemoradiotherapy; [2]Adjuvant chemoradiotherapy; [3]3-year OS; [4]20% of patients enrolled had AC of the gastroesophageal junction (GEJ). MS: Median survival; SCC: Squamous cell carcinoma; AC: Adenocarcinoma; B: Bleomycin; C: Cisplatin; F: Fluorouracil; V: Vindesine; NS: Not significant; OS: Overall survival.

With such inconclusive and often contradictory results in trials that have evaluated neoadjuvant multimodality treatment based on disparate study populations, a myriad of regimen protocols, and more importantly, small numbers of patients, numerous meta-analyses have subsequently been performed in an effort to synthesize these data into larger pools and discover if a survival benefit exists[66,83-87]. One of the first, published by Urshel and Vasan, included nine randomized controlled trials with 1116 patients and reported a 3-year survival benefit that favored neoadjuvant chemoradiotherapy (OR: 0.66, 95% CI: 0.47-0.92, $P = 0.016$), which was most pronounced when the chemotherapy and radiotherapy were given concurrently (OR: 0.45, 95% CI: 0.26-0.79, $P = 0.005$) instead of sequentially (OR: 0.82, 95% CI: 0.54-1.25, $P = 0.36$). Although patients who received neoadjuvant chemoradiotherapy were less likely to proceed to surgery (OR: 2.50, 95% CI: 1.05-5.96, $P = 0.038$), they were still more likely to have an R0 resection (OR: 0.53, 95% CI: 0.33-0.84, $P = 0.007$) with 21% having a pCR. Although there was a decreased risk of local-regional recurrence for those who received multimodality treatment compared to those who received surgery alone (OR: 0.38, 95% CI: 0.23-0.63, $P = 0.0002$), there was no difference in risk for distant recurrence. There was a statistically insignificant but nonetheless concerning trend toward increased treatment mortality (OR: 1.63, 95% CI: 0.99-2.68, $P = 0.053$)[84]. The most recent meta-analysis published by Gebski and colleagues has evaluated 1209 patients in 10 trials, and likewise found a statistically significant benefit with neoadjuvant chemoradiotherapy compared to surgery alone, with a 19% decreased risk of death (HR: 0.81, 95% CI: 0.70-0.93, $P = 0.002$) for both AC and SCC, which corresponded to a 13% absolute difference in survival at 2 years[66].

As noted earlier, Gebski *et al*[66] also have evaluated neoadjuvant chemotherapy compared to surgery alone in a meta-analysis. These separate meta-analyses have been published at the same time in conjunction with each other. Although the two neoadjuvant chemotherapy and chemoradiotherapy data pools are not directly comparable, the absolute survival benefit of chemotherapy appears to be less than that of chemoradiotherapy (7% *vs* 13% at 2 years). This point was further supported although not confirmed by Stahl *et al*[88] who randomized 126 patients with AC of the GEJ (55% were type I GEJ tumors) to 16 wk neoadjuvant chemotherapy using cisplatin and leucovorin-modulated fluorouracil, or 12 wk of the same regimen followed by 3 wk of cisplatin and etoposide with concurrent radiotherapy (30 Gy) prior to surgical resection. Those treated with multimodality neaodjuvant chemoradiotherapy did not have a significant increase in R0 resection (72% *vs* 70%), but did have an increased probability of achieving a pCR (15.6% *vs* 2%, $P = 0.03$) and having tumor-free lymph nodes at the time of resection (64% *vs* 38%, $P = 0.01$) compared to those treated with neoadjuvant chemotherapy. There was a trend toward improved 3-year OS (47% *vs* 28%, $P = 0.07$), which favored neoadjuvant chemoradiotherapy, but with just a third of the expected 354 patients enrolled in the trial prior to its closure due to poor accrual, there was no statistically significant difference noted.

Anecdotally, patients with esophageal cancer often lack the strength to complete adjuvant chemoradiotherapy, although there are data to support its use and tolerability in patients with tumors of the GEJ[82]. The U.S. INT 0116 trial enrolled 556 patients with resected AC of the stomach and GEJ; approximately 20% of those participating had GEJ tumors. Patients were randomized to either sur-

gery alone or surgery followed by four cycles of adjuvant leucovorin-modulated fluorouracil, with the second cycle concurrent with radiotherapy (45 Gy). The median OS was 27 mo *vs* 36 mo (HR: 1.35, 95% CI: 1.09-1.66, P = 0.005), which favored the adjuvant chemoradiotherapy arm. Although 17% of patients were unable to finish the protocol because of treatment-related toxicity, an impressive 64% of patients were able to finish the protocol completely. There was no difference in survival based on the location of the primary tumor[82].

Targeted therapy

Despite improvements seen with the multimodality treatment of esophageal cancer, cure rates remain disappointingly low[66]. As such, targeted agents that have been found to benefit patients with head and neck, breast, lung, colon, and pancreatic cancers have generated intense interest in esophageal cancer[89-91]. Multiple pathways have been evaluated at the molecular level with potential targets in esophageal cancer including cyclin-dependent kinases, nuclear factor κB, matrix metallopreoteinases, and the inhibition of *COX-2*. The most promising targets at present, however, appear to be the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF)[89].

There are at least four types of EGFR: EGFR (human EGFR-1, HER-1), HER-2, HER-3, and HER-4. EGFR signaling plays a crucial role in modulating cell proliferation, invasion, metastasis, and resistance to cell death[89]. Overexpression of EGFR proteins has been reported in 30%-70% of AC and SCC of the esophagus, with such overexpression correlating with more aggressive disease and worse outcome[92-94]. Multiple clinical trials have been launched in an effort to target EGFR in esophageal cancer, with the most common drug used being the IgG1 monoclonal antibody cetuximab[95-99]. A trial by Gold *et al*[95] using cetuximab as a second-line monotherapy in the metastatic setting was discouraging, although regimens using cetuximab in combination with FOLFIRI[96], cisplatin and docetaxel[97], and cisplatin and fluorouracil[98] have revealed that the drug shows promise in the treatment of esophageal cancer. A phase Ⅱ trial by Safran *et al*[99] has evaluated 57 patients with esophageal cancer that were treated with weekly carboplatin, paclitaxel and cetuximab with concurrent radiotherapy (50.4 Gy). Seventy percent of patients achieved a complete clinical response and, of the 49 patients who went on to surgery, 27% had a pCR. The RTOG 0436 trial - a phase Ⅲ trial that is evaluating carboplatin, paclitaxel, and concurrent radiotherapy with or without cetuximab - is currently ongoing.

Another EGFR that is more famously associated with breast cancer, HER-2, is also overexpressed in 19%-43% of patients with esophageal cancer, and can be targeted by trastuzumab - a humanized IgG1 monoclonal antibody against the same receptor[100]. The phase Ⅲ ToGA trial randomized 594 patients with locally advanced, recurrent, or metastatic gastroesophageal cancer with HER-2 overexpression to treatment with cisplatin and fluorouracil or capecitabine, with or without trastuzumab. The median OS was significantly improved and favored the arm that received trastuzumab (13.5 mo *vs* 11.1 mo, HR: 0.74, 95% CI: 0.60-0.91, P = 0.0048)[101]. How these results will affect future multimodality neoadjuvant treatment is unknown, especially considering the potential for cardiotoxicity in a patient population that is already at risk. Although there were no differences in symptomatic congestive heart failure between the two arms, the patients who received trastuzumab were more likely to experience asymptomatic decreases in their left ventricular ejection fraction (4.6% *vs* 1.1%)[101].

VEGF is a regulator of angiogenesis and is yet another potential target. Similar to EGFR, VEGF is also overexpressed in 30%-60% of esophageal cancer patients and is likewise associated with poor outcome[102]. There is even evidence to suggest that the level of VEGF expression increases during treatment with chemotherapy and radiotherapy, which makes it a particularly attractive target for multimodality neoadjuvant treatment[103,104]. Promising phase Ⅱ data with surgically unresectable AC of the GEJ combining bevacizumab - a humanized monoclonal antibody against VEGF - with cisplatin and irinotecan[105], as well as docetaxel, cisplatin and fluorouracil[106] are available, while trials that are incorporating neoadjuvant chemoradiotherapy with the addition of bevacizumab are currently ongoing[91]. As with trastuzumab, it is unknown how the potential toxicities inherent to bevacizumab - hypertension, thromboembolism, poor wound healing, bowel perforation, worsening arterial disease, and an increased risk of bleeding - will affect the treatment of esophageal cancer patients who often present with multiple comorbidities[107].

CONCLUSION

The optimal treatment strategy for resectable esophageal cancer is still a controversial topic. Multimodality neoadjuvant chemotherapy with concurrent radiotherapy has been accepted by many - although not all - as the standard of care, because such a regimen increases rates of pCR, R0 resection, and local tumor control, which all correlate with improved OS[33,66,77,78,81,84-86]. If one accepts the most recent meta-analysis, an absolute OS benefit exists but is likely to be just 13% at 2 years[66]. With such a small benefit, it is no wonder that the multiple underpowered clinical trials that have compared neoadjuvant chemoradiotherapy with surgery alone have found it difficult to demonstrate a survival difference.

Although such a survival benefit might seem small, it should be noted that it is in line with accepted treatment algorithms of other lethal malignancies, such as the addition of adjuvant chemotherapy in completely resected non-small cell lung cancer[108]. The need to treat approximately eight patients with a difficult-to-tolerate regimen to cure just one additional person is hardly ideal, yet these odds are not inconsequential when discussing them face-to-face with a patient who is at least felt to be sufficiently medically fit enough to withstand an esophagectomy.

Although neoadjuvant and perioperative chemother-

apy have also been found to be effective approaches for treating esophageal cancer, there is a reasonable amount of evidence to support the notion that such treatments are inferior to neoadjuvant chemoradiotherapy[66,88], while the data supporting adjuvant chemoradiotherapy can only be applied to patients with GEJ tumors at the present time[82]. How targeted therapy will affect our approach to resectable esophageal cancer is currently unknown as many of the trials to determine this are ongoing[91,99]. By participating in clinical trials and enrolling as many appropriate patients as we possibly can, these questions will hopefully be answered in a more timely and conclusive manner than previously seen in the history of esophageal cancer treatment.

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TOPIC HIGHLIGHT

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Transhiatal *versus* transthoracic esophagectomy for esophageal cancer

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Abstract

Esophageal cancer continues to represent a formidable challenge for both patients and clinicians. Relative 5-year survival rates for patients have improved over the past three decades, probably linked to a combination of improved surgical outcomes, progress in systemic chemotherapy and radiotherapy, and the increasing acceptance of multimodality treatment. Surgical treatment remains a fundamental component of the treatment of localized esophageal adenocarcinoma. Multiple approaches have been described for esophagectomy, which can be thematically grouped under two major categories: either transthoracic or transhiatal. The main controversy rests on whether a more extended resection through thoracotomy provides superior oncological outcomes as opposed to resection with relatively limited morbidity and mortality through a transhiatal approach. After numerous trials have addressed these issues, neither approach has consistently proven to be superior to the other one, and both can provide excellent short-term results in the hands of experienced surgeons. Moreover, the available literature suggests that experience of the surgeon and hospital in the surgical management of esophageal cancer is an important factor for operative morbidity and mortality rates, which could supersede the type of approach selected. Oncological outcomes appear to be similar after both procedures.

Key words: Esophageal cancer; Transhiatal esophageal resection; Transthoracic esophageal resection

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INTRODUCTION

Esophageal cancer is the eighth most common cancer worldwide, with a wide variation in its frequency between high- and low-incidence regions. There are two main histopathological subtypes: squamous cell carcinoma (SCC) and adenocarcinoma. SCC is the most common subtype in several endemic regions of the world[1], with a high correlation to smoking and alcohol abuse, as well as chronic inflammation[2]. On the other hand, adenocarcinoma is commonly associated with Barrett's metaplasia, gastroesophageal reflux disease (GERD), and obesity[3]. It has become the most common subtype in the western hemisphere, and frequently involves the gastroesophageal junction (GEJ) and proximal stomach. SCC and adenocarcinoma of the esophagus are distinct entities and should be considered as such when defining optimal therapy. As

a result of its increasing incidence[4] and relationship with GERD, the following review focuses on adenocarcinoma of the esophagus.

Despite improvements in systemic chemotherapy and radiotherapy, and the increasing acceptance of multimodality treatment that have resulted in enhanced survival rates over the past three successive decades[5], surgical resection continues to be the mainstay of care for treatment of localized esophageal adenocarcinoma. Multiple approaches have been described for esophagectomy, and they can be thematically categorized under two major headings: transthoracic or transhiatal. The transthoracic procedure is performed more commonly by means of combined laparotomy and right thoracotomy (Ivor Lewis procedure). Other options include left thoracotomy with or without cervical incision, a single left thoracoabdominal incision, or a three-incision resection with a cervical anastomosis (McKeown procedure). The transhiatal approach is performed through midline laparotomy and left cervical incision. There has been considerable controversy about which procedure provides the best short- and long-term outcomes. The discussion centers around whether more extended resection through thoracotomy provides superior oncological outcomes than resection with relatively limited morbidity and mortality through a transhiatal approach. Decisions regarding surgical technique are frequently based on personal bias, surgeons' experience and comfort with a procedure. The issue of the extent of surgical resection is addressed first, with a brief description of each approach. The relevance of surgeon/hospital volume and its relationship with adequate outcomes after esophagectomy, and the role of surgery in the context of multimodality treatment are discussed separately.

TRANSTHORACIC ESOPHAGECTOMY

Transthoracic esophagectomy is most commonly performed *via* laparotomy followed by right thoracotomy and intrathoracic anastomosis (Ivor Lewis procedure). It was originally described in 1946 in two stages[6], and historically, it is the standard procedure against which all other techniques are measured. Left thoracotomy or thoracoabdominal incision provides adequate exposure to the distal esophagus, but presents greater difficulty to access the upper and middle thirds and to perform an anastomosis high in the chest.

Ivor Lewis esophagectomy starts through a midline incision in the abdomen. The left lobe of the liver is mobilized and retracted laterally, and the stomach is fully mobilized and freed from its vascular attachments, including an upper abdominal lymphadenectomy, while preserving the right gastroepiploic and right gastric vessels on whose pedicle the reconstructive conduit is based. The duodenum is mobilized completely *via* a Kocher maneuver and a pyloric drainage procedure is performed, to diminish gastric stasis and minimize aspiration[7,8]. The right diaphragmatic crus is divided with electrocautery to allow access to the mediastinum and to avoid constricting the transposed stomach. Placement of a feeding jejunostomy is commonly performed before abdominal closure and repositioning for the thoracic component of the procedure. Muscle-sparing right lateral thoracotomy is then performed through the fifth intercostal space. The mediastinal pleura that overlies the esophagus is incised, the azygos vein is divided, the intrathoracic esophagus is mobilized, and *en bloc* resection of the surrounding periesophageal tissue is performed, including mediastinal lymph node dissection.

After division of the proximal esophagus in the chest to ensure an adequate margin, the GEJ and stomach are transposed into the thoracic cavity. A gastric conduit is now created, with a linear stapler parallel to the greater curve, and the fundus is removed with a portion of the lesser curvature. The specimen is removed, and an esophagogastric anastomosis is performed. The McKeown procedure is an alternative three-incision approach, in which right thoracotomy is the initial stage of the procedure, followed by repositioning of the patient in the supine position for abdominal and left cervical incision, to achieve a cervical esophagogastric anastomosis.

The theoretical advantage of the transthoracic approach is a more thorough oncological operation as a result of direct visualization and exposure of the thoracic esophagus, which allows a wider radial margin around the tumor and more extensive lymph node dissection. However, the combined effects of an abdominal and thoracic incision might compromise cardiorespiratory function, especially in patients with coexisting lung or heart disease. The other disadvantage is that an intrathoracic anastomotic leak can lead to catastrophic consequences including mediastinitis, sepsis, and death. The three-incision modification of the procedure effectively eliminates the potential for complications associated with an intrathoracic esophagogastric anastomosis.

The perioperative mortality of transthoracic esophagectomy in experienced centers ranges from 9% to as low as 1.4%[9-15]. Five-year survival in approximately 25% of patients who undergo transthoracic esophageal resection has been reported. These reports include heterogeneous populations of patients with esophageal cancer that underwent a variety of surgical approaches, the use of adjuvant treatment in some but not all patients, and combined histologies (SCC and adenocarcinoma).

TRANSHIATAL ESOPHAGECTOMY

Transhiatal esophagectomy was first performed by Turner in 1933 for esophageal carcinoma[16]. During subsequent decades, it was not routinely performed since the transthoracic approach was preferred after general anesthesia became available. In 1978, Orringer described his initial series of blunt transhiatal esophagectomy, which kindled new interest in this procedure[17]. It has gained favor among surgeons concurrent with the rising incidence of adenocarcinoma of the distal esophagus, which is readily approachable through the diaphragmatic hiatus.

The abdominal portion of the procedure duplicates that of the previously described transthoracic approach

and includes mobilization of the stomach, pyloromyotomy and placement of a feeding jejunostomy. Again, cautery division of the right crus allows access to the mediastinum and dissection under direct vision of the distal and middle third of the esophagus. A left cervical incision along the anterior border of the sternocleidomastoid muscle provides exposure to the cervical esophagus. Circumferential dissection of the cervical esophagus is carried down to below the thoracic inlet, and blunt dissection is continued into the superior mediastinum to mobilize the upper thoracic esophagus, with care to avoid injury to the recurrent laryngeal nerve in the tracheoesophageal groove. The remainder of the dissection at the level of and superior to the carina is completed by blunt dissection through the esophageal hiatus. The cervical esophagus is then divided, the stomach and attached intrathoracic esophagus are delivered through the abdominal wound, and a gastric conduit is fashioned using a linear stapling device in the same manner as described above. The gastric tube is delivered through the posterior mediastinum to the cervical wound, where a cervical esophagogastric anastomosis is performed. The stomach is considered by most surgeons as the ideal replacement for the resected esophagus, although a segment of colon or a free flap of small bowel can be used as alternative conduits[18,19].

The postulated advantages of the transhiatal approach to esophagectomy are avoidance of a thoracotomy incision, which thereby minimizes pain and subsequent postoperative pulmonary complications; elimination of potentially life threatening mediastinitis as a result of an intrathoracic anastomotic leak; and a shorter duration of operation, which potentially results in decreased morbidity and mortality[17]. Leak of a cervical esophagogastric anastomosis can be handled in the vast majority of patients with opening of the cervical wound, followed by local wound care. Compared to transthoracic esophagectomy, transhiatal esophagectomy is associated with poor visualization of upper and middle thoracic esophageal tumors (potentially compromising the oncological integrity of the operation), increased anastomotic leak rate with subsequent stricture formation, and a higher risk of recurrent laryngeal nerve injury[20,21].

The reported postoperative mortality after transhiatal esophagectomy in individual series tends to be slightly lower than that of the transthoracic approach, between 1% and 7.5%[22-26], and 5-year survival rate is approximately 25%, which is not substantially different from that accomplished after the transthoracic approach. Orringer *et al*[26] have reported the most extensive experience with transhiatal esophagectomy. Their latest report involved 2007 patients, of which 1525 had a diagnosis of cancer. Seventy-two percent had adenocarcinoma, and 38% received neoadjuvant chemoradiation, with a 5-year survival rate of 29%. Among this series, their most recent group of 944 patients had a hospital mortality of 1%. The anastomotic leak rate was 9% in this same group, and recurrent laryngeal nerve injury occurred in 2% of cases. These results reflect those reported from other surgical series of transhiatal esophagectomy.

Table 1 Meta-analysis comparing transthoracic and transhiatal esophagectomy

Meta-analysis	Rindani *et al*[20]	Hulscher *et al*[21]
No. of patients	5483	7527
Postoperative mortality (%)		
TT	9.5	9.2
TH	6.3	5.7
Intraoperative blood loss (mL)		
TT	1171	1001
TH	1311	728
Hospital stay (d)		
TT	19.8	21
TH	19.5	17.8
Pulmonary complications (%)		
TT	25	18.7
TH	24	12.7
Cardiac complications (%)		
TT	10.5	6.6
TH	12.4	19.5
Anastomotic leakage (%)		
TT	10	7.2
TH	16	13.6
Vocal cord paralysis (%)		
TT	4.8	3.5
TH	11.2	9.5
5-yr OS (%)		
TT	26	23
TH	24	21.7

TT: Transthoracic esophagectomy; TH: Transhiatal esophagectomy; OS: Overall survival.

STUDIES COMPARING TRANSTHORACIC *VS* TRANSHIATAL ESOPHAGECTOMY

The question of one approach being superior to the other continues to generate considerable controversy among surgeons. No definitive advantage in oncological outcome or postoperative morbidity and mortality can be concluded from the non-comparative case series mentioned above.

Two large meta-analyses have addressed these issues by utilizing collective reviews of numerous individual studies that have compared transhiatal esophagectomy to transthoracic esophagectomy[20,21]. Most of the studies included in these meta-analyses were retrospective in nature and were not consistent with respect to the surgical technique utilized and which therapy in addition to surgery was delivered. Nevertheless, the results of both were very similar.

The meta-analysis by Rindani *et al*[20] included almost 5500 patients from 44 series published between 1986 and 1996 (Table 1). The statistical analysis was descriptive rather than comparative due to the diverse nature of the series, and there was only one prospective randomized trial included, with a small sample and short follow-up. Postoperative respiratory and cardiovascular complications were almost identical between the two groups. The transhiatal group had a higher incidence of anastomotic leaks and recurrent laryngeal nerve injuries. Thirty-day mortality was 6.3% after transhiatal and 9.5% after transthoracic resection, but survival at 5 years was equivalent between the two procedures.

The second meta-analysis, by Hulscher *et al*[21], involved

Table 2 Randomized trials comparing transthoracic and transhiatal esophagectomy

Meta-analysis	Goldminc *et al*[27]	Chu *et al*[28]	Jacobi *et al*[29]	Hulscher *et al*[30,31]
No. of patients	67	39	32	220
Postoperative mortality (%)				
TT	8.6	0	6	4
TH	6.2	0	6	2
Intraoperative blood loss (mL)				
TT	[1](2.3 units transfused)	671	2270	1900
TH	[1](2.3 units transfused)	724	1000	1000
Hospital stay (d)				
TT	18	27	21	19
TH	20.5	18	23	15
Postoperative pneumonia (%)				
TT	20	0	31	57 (atelectasis included)
TH	19	10	19	27 (atelectasis included)
Cardiac complications (%)				
TT	[1]	15.8	19	26
TH	[1]	15	31	16
Anastomotic leakage (%)				
TT	9	0	12.5	16 (subclinical included)
TH	6	0	12.5	14 (subclinical included)
Vocal cord paralysis (%)				
TT	3	[1]	[1]	21 (transient)
TH	3	[1]	[1]	13 (transient)
Reported survival (%)				
TT	22 at 3 yr	Median survival 13.5 mo	77 at 1 yr	36 at 5 yr
TH	30 at 3 yr	Median survival 16 mo	70 at 1 yr	34 at 5 yr

[1]Data not reported or did not occur. TT: Transthoracic esophagectomy; TH: Transhiatal esophagectomy.

over 7527 patients derived from 50 studies from 1990 to 1999 (Table 1). Six were prospective comparative studies, three of which were randomized, all with a relatively small sample size. None of these three studies could demonstrate a significant difference in morbidity, mortality, or long-term survival[27-29]. When all 50 studies were analyzed, no significant differences were demonstrated in the overall morbidity rate. Blood loss was higher after transthoracic esophagectomy, and it had a higher risk of pulmonary complications, chylous leakage (2.4% *vs* 1.4%) and wound infection (7.7% *vs* 4.3%). Similar to the previous meta-analysis, transhiatal esophagectomy had a higher incidence of anastomotic leakage and recurrent laryngeal nerve injury. Length of stay in the intensive care unit (ICU) and hospital were longer in the transthoracic group, and in-hospital mortality was significantly higher as well. Again, there was no difference in 5-year survival rates.

There have been a total of four randomized trials that have compared both techniques (Table 2). Three of them, included in the previous meta-analyses described above, could not provide definitive conclusions and each was hampered by an extremely small sample size, with non-significant differences reported between the two arms[27-29].

The fourth randomized trial, published in 2002 by Hulscher *et al*[30], has provided level I evidence regarding this controversial issue. Two hundred and twenty patients were assigned to either transhiatal or transthoracic esophagectomy with cervical anastomosis. The transthoracic esophagectomy procedure included *en bloc* resection of the thoracic duct, azygos vein, ipsilateral pleura, and all peri-esophageal tissue in the mediastinum, including a formal lymphadenectomy. Transhiatal esophagectomy had a shorter operative duration than transthoracic esophagectomy (3.5 h *vs* 6 h), with lower blood loss (1 L *vs* 1.9 L). Perioperative morbidity rate was also lower in the transhiatal group (pulmonary complications, 57% *vs* 27%; chylous leakage, 10% *vs* 2%). Duration of mechanical ventilation, ICU stay and hospital stay were all shorter in the transhiatal group. However, there was no significant difference in hospital mortality (transthoracic: 4%; transhiatal: 2%). Although initially a trend toward a survival benefit with transthoracic approach was seen, after longer follow-up, no difference in 5-year overall survival was found (transthoracic: 36%; transhiatal 34%). Notably, the transthoracic approach was of benefit in some subgroups; patients with 1-8 positive lymph nodes had better disease-free survival rate (64% *vs* 23%), and patients with tumors arising from the distal esophagus (rather than gastric cardia) tended towards a survival benefit (51% *vs* 37%, not statistically significant)[31]. However, this phase Ⅲ study was not adequately powered to address these subgroup analyses.

A large population-based study has been published recently, which has evaluated the results of both approaches through the Surveillance, Epidemiology and End Results (SEER) - Medicare linked database from 1992 to 2002[32]. A lower operative mortality was found after transhiatal esophagectomy (6.7% *vs* 13.1%). Although observed 5-year survival was higher after transhiatal esophagectomy, after adjusting for stage, patient and provider factors, no significant 5-year survival difference was found.

These data suggest that perioperative and oncological outcomes are not substantially influenced by the surgical approach to esophagectomy, and that either procedure is associated with acceptable results in the hands of expe-

rienced surgeons. Ideally, surgeons and hospitals treating patients with esophageal carcinoma should have expertise in both techniques. Some patients might even benefit from an individualized approach. For an older or higher-risk surgical patient, for whom perioperative recovery is an even greater concern than usual, a transhiatal approach could confer an advantage. In a fit patient with evidence of a limited number of involved lymph nodes, there is some evidence (although not level I evidence) that suggests a benefit in survival with the transthoracic approach. Still, available literature suggests that experience of the surgeon and hospital is likely to be a more important factor than is the type of approach selected.

SURGEON/HOSPITAL VOLUME AND ESOPHAGECTOMY

There is increasing evidence that confirms that patients who undergo complex oncological resections, such as esophagectomy, at high-volume hospitals by experienced surgeons have significantly lower rates of perioperative morbidity and mortality[33-35]. This association has been shown for several surgical procedures in studies that have used health-services-linked databases. However, the association between volume and outcome for esophagectomy is one of the strongest among all complex cancer operations[33-35]. Furthermore, a recent analysis of the SEER - Medicare linked data base[36] suggests that long-term survival, and therefore oncological outcome, is also volume dependent. The probability of surviving 5 years following esophagectomy in high-volume hospitals was 34%, whereas 5-year survival probability in low-volume hospitals was only 17%. This 17% absolute difference in 5-year survival following esophagectomy between high-volume and low-volume hospitals was the highest amongst all cancer resections surveyed. Volume-dependent discrepancy in 5-year survival could not be attributed to differences in the delivery of adjuvant therapy. Therefore, not only are short term procedure-related outcomes associated with surgical experience but long-term oncological outcomes might also be affected by surgeon and center volume/experience with esophageal resection. The basis for this improved survival has not been defined and requires further investigation.

ROLE OF SURGERY IN THE MULTIMODALITY THERAPY ERA

Relative 5-year survival rates for patients with esophageal cancer have improved over the past three successive decades[5,37]. The reasons for this trend are surely multifactorial and could include the widespread acceptance and use of a multimodality treatment approach, improved surgical outcomes, and progress made in systemic chemotherapy and radiotherapy.

Based on the current level I evidence, it can be reasonably argued that the addition of surgery to an effective regimen of chemoradiotherapy in patients with SCC of the esophagus might not improve outcome. Two randomized trials have addressed the role of chemoradiotherapy alone *vs* chemoradiotherapy followed by surgery in patients with SCC. The German Esophageal Cancer Study Group[38] has demonstrated better 2-year local, progression-free survival in the surgical group (64.3% *vs* 40.7%), although with increased treatment-related mortality (12.8% *vs* 3.5%), and equivalent overall survival between the two treatment groups. The FFCD 9102 trial[39], in which 90% of the patients had a diagnosis of SCC, found a higher frequency of locoregional relapse in the chemoradiotherapy alone group, but with a lower 3 mo mortality rate. As in the German study, survival rates were similar in both groups.

In contrast to SCC, the controversy regarding patients with esophageal adenocarcinoma has been centered on the added value of preoperative combined modality therapy, and not the necessity of surgical resection. Despite the fact that numerous phase Ⅲ trials[14,40-43] have compared preoperative chemoradiotherapy followed by surgery to surgery alone, it is not clear that preoperative chemoradiotherapy can be declared as a standard of care.

One randomized trial from Ireland[41] has shown a benefit in patients with adenocarcinoma, but definitive conclusions are hampered by the small sample size, unusually poor results with surgery alone, and short follow-up. More recently, the Cancer and Leukemia Group B initiated a trial that was closed prematurely due to poor accrual[43]. The most common histological tumor subtype in this study was adenocarcinoma. Reported median survival (4.48 years *vs* 1.79 years) and 5-year survival (39% *vs* 15%) favored trimodality therapy. Its major limitation was the incredibly small patient sample size due to poor accrual, although the findings had statistical significance.

Although the survival benefits have not been consistent, the majority of patients are down-staged with preoperative chemoradiotherapy, and for those patients who have a substantial response (complete pathological or major partial response defined by residual microscopic disease in the resected specimen) to preoperative chemoradiotherapy, there is a survival advantage. Surgery appears to be a crucial component of combined modality therapy to eliminate residual disease following chemoradiotherapy that leads to improved locoregional control and improved long-term survival. However, failure at a distant site is common and is the most frequent cause of death.

Even though the evidence for the benefit of preoperative chemoradiotherapy in the treatment of patients with esophageal cancer is not compelling, the combined modality approach has gained acceptance in most centers in the United States, and is by far the most frequent therapeutic option offered to patients with cancer of the esophagus. A meta-analysis has reported that preoperative chemoradiotherapy improved 3-year survival by 13% over surgery alone with similar improvement identified in patients with either SCC or adenocarcinoma histology[44]. Although the role of surgery has been questioned, especially for SCC, it can be reasonably concluded that esophageal resection remains an important, if not the most impor-

tant, therapeutic component of a combined modality approach to esophageal cancer. However further refinement of our treatment of patients with esophageal cancer is warranted. Patients who achieve a complete pathological response to combined chemoradiotherapy probably will obtain no advantage from undergoing esophagectomy, considering the substantial morbidity and mortality associated with the procedure. Unfortunately, current diagnostic methods are not reliable to identify this group of patients preoperatively. In contrast, it is reasonable to expect that patients with residual disease, either apparent or occult, following preoperative combined modality treatment will benefit from eradicating that residual disease with resection to give them the best opportunity for a long-term disease-free state. Surgeons interested in this lethal disease should direct their efforts to more accurate identification of those patients that will likely benefit from different single or combination treatment modalities, and tailor their therapeutic interventions accordingly.

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TOPIC HIGHLIGHT

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Minimally invasive esophagectomy

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Abstract

Esophageal resection is associated with a high morbidity and mortality rate. Minimally invasive esophagectomy (MIE) might theoretically decrease this rate. We reviewed the current literature on MIE, with a focus on the available techniques, outcomes and comparison with open surgery. This review shows that the available literature on MIE is still crowded with heterogeneous studies with different techniques. There are no controlled and randomized trials, and the few retrospective comparative cohort studies are limited by small numbers of patients and biased by historical controls of open surgery. Based on the available literature, there is no evidence that MIE brings clear benefits compared to conventional esophagectomy. Increasing experience and the report of larger series might change this scenario.

Key words: Esophageal resection; Transhiatal esophagectomy; Transthoracic esophagectomy; Esophageal cancer; Minimally invasive esophagectomy; Laparoscopy; Thoracoscopy

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INTRODUCTION

Esophageal cancer is a devastating disease. It was estimated in 2002 that 462 117 individuals developed the disease and 385 892 died worldwide[1], which corresponds to a mortality rate of 83.5%. Surgery has been considered an essential part of the treatment of patients with esophageal adenocarcinoma. However, surgery has been traditionally associated with a high morbidity and mortality rate. A lot of progress has been made since Earlam and Cunha-Melo in 1980 reviewed the literature and reported 29% mortality for esophagectomy[2]. Recent series have shown much improved rates, but they are still far from ideal. For these reasons, minimally invasive esophagectomy (MIE) brought high hopes to this field.

This final paper from a seminar on heartburn and adenocarcinoma focuses on the minimally invasive approach to esophagectomy; a treatment that is suitable for Barrett's esophagus and high-grade dysplasia and for esophageal adenocarcinoma.

TECHNIQUE

The techniques for esophagectomy can be simplistically described as those that include thoracotomy (transthoracic) and those without thoracotomy (transhiatal). The same classification can be used for MIE. According to the preferred approach, thoracotomy can be replaced by thoracoscopy and/or laparotomy can be replaced by laparoscopy. Thus, the following different combinations can be found in the literature: (1) transhiatal esophagectomy -

laparoscopy and cervicotomy[3,4]; (2) transthoracic esophagectomy (three-field) - laparoscopy, thoracoscopy and cervicotomy[5,6]; (3) transthoracic esophagectomy (three-field) - laparotomy, thoracoscopy and cervicotomy[6]; (4) transthoracic esophagectomy (three-field) - laparoscopy, thoracotomy and cervicotomy[7]; (5) transthoracic esophagectomy (Ivor Lewis) - laparoscopy and thoracoscopy[5]; (6) transthoracic esophagectomy (Ivor Lewis) - laparotomy and thoracoscopy[8]; and (7) transthoracic esophagectomy (Ivor Lewis) - laparoscopy and thoracotomy[9].

Laparoscopy

The laparoscopic approach to esophagectomy has the purpose of: (1) dissection of the abdominal esophagus and esophageal hiatus; (2) abdominal lymphadenectomy; (3) preparation of the stomach to replace the esophagus; (4) pyloroplasty or pyloromyotomy; and (5) placement of a feeding jejunostomy.

Dissection of the abdominal esophagus and esophageal hiatus follows the same principles of laparoscopic antireflux surgery. In summary, five abdominal ports are usually used. The abdominal esophagus and esophageal hiatus are dissected. The gastro-hepatic ligament is open, which preserves the right gastric artery. The greater curvature of the stomach is mobilized, which preserves the right gastroepiploic artery. The left gastric artery and coronary vein are isolated and divided with an endo-GIA stapler. A gastric conduit is constructed by dividing the stomach, starting on the lesser curvature and finishing at the angle of His. Pyloroplasty or pyloromyotomy is usually performed. The tip of the gastric conduit is sutured to the esophageal specimen that is retrieved through the neck or through the thorax if the anastomosis is performed in the chest[5,9,10]. Alternatively, the gastric conduit might be created through a mini-laparotomy[11]. The colon is rarely used for esophageal replacement during MIE.

Extended abdominal lymphadenectomy might be added to the procedure based on the philosophy adopted for the treatment of esophageal cancer by the surgeon. It is safe and feasible with a laparoscopic approach, after the lessons learned with laparoscopic treatment of gastric cancer[12].

Thoracoscopy

The thoracoscopic approach to esophagectomy has the purpose of: (1) dissection of the thoracic esophagus; (2) thoracic lymphadenectomy; and (3) esophageal anastomosis.

Dissection of the esophagus is performed using four ports in the right chest. Carbon dioxide insufflation is not considered necessary by most surgeons. The deflated lung is retracted anteriorly and the mediastinal pleura overlying the esophagus is divided. The azygos vein is then divided using an endo-GIA stapler with a vascular cartridge. A Penrose drain is placed around the esophagus to facilitate retraction. The esophagus is circumferentially mobilized from the esophageal hiatus up to the thoracic inlet. An esophageal anastomosis might be performed above the level of the azygos vein with the aid of a linear stapler. Otherwise, once the thoracoscopic dissection is completed, the operation can continue with cervicotomy, and the continuity of the digestive tract is restored with transposition of the stomach to the neck[5,9,10].

Similarly to the laparoscopic approach, extended mediastinal lymphadenectomy might be performed.

TECHNICAL VARIATIONS

Hand-assisted esophagectomy

Some surgeons perform transhiatal MIE using a laparoscopic approach to the abdomen but include a subxiphoid midline incision for manual mobilization of the mediastinal esophagus through a hand-port[5].

Prone position

Some surgeons have proposed a prone position for thoracoscopy instead of a left lateral decubitus approach[13,14]. This approach is used in order to improve ergonomics, operative time and pulmonary complications. The patient is placed in the prone position and the esophagus is approached through the right chest. The right lung is kept ventilated but it is collapsed due to the action of gravity and an 8-mmHg CO_2 pneumothorax[13,14].

Palanivelu *et al*[13] have reported an incidence of 2% for pleural and pulmonary complications in 130 patients. Fabian *et al*[15] have shown no differences in blood loss, number of lymph nodes dissected, and complications in two small cohorts of patients operated in left lateral decubitus *vs* prone position. However, operation time was significant shorter. Although good results have been reported, this technique is not widely accepted.

Robotic surgery

Robotic surgery claims to have the advantages of: (1) eliminating the counter-intuitive motion of standard laparoscopy; (2) aligning the eyes and hands over the area of interest with improved ergonomics; (3) increasing freedom of instrument movement by allowing wrist and finger movements that standard laparoscopic instruments do not have; (4) minimizing instrument tremor; and (5) 3D stereoscopic vision with dual camera technology[16]. Different types of esophageal operations have been performed with the aid of a robotic platform. Cases of robotic esophagectomy have been shown to be safe and feasible, either through thoracoscopy[17] or laparoscopy[4].

Early results have shown a conversion rate ranging from 0% to 15%[4,18]. Operating time is still high for transthoracic robotic esophagectomy, at an average of 7.5 h, which leads to a high incidence of pulmonary complications that decreases with experience[18]. Long-term outcomes are still elusive.

Vagal-sparing esophagectomy

Vagal-sparing esophagectomy is an attractive alternative to conventional procedures to avoid postoperative complications associated with vagotomy. Vagal-sparing

MIE has been described and popularized by the Portland Group[19]. The technique follows the same principles as open surgery: the vagal nerves are mobilized off the distal esophagus and stomach to the level of the pylorus; two nasogastric tubes are passed distally through the cervical esophagus and into the gastric remnant; the gastric remnant is divided and the nasogastric tubes are incorporated into the staple line; and finally, the esophagus is inverted, stripped out and removed through the cervicotomy[19].

OUTCOMES

Intraoperative complications are still frequent and they are the main cause for conversion to open surgery. During laparoscopy, bleeding is the main complication, either at the splenic hilum or parenchyma (often requiring splenectomy) or during division of gastric vessels at the time of the preparation of the gastric conduit[5]. Liver injury has also been reported[6,20]. During thoracoscopy, bleeding is reported as well[6]; however, the presence of pleural adhesions is the main cause for conversion[5,6]. Overall, the conversion rate ranges from 3% to 18%[5,6] with an average of 5%-7% depending on the technique[21,22].

Postoperative complications average 40%-50%, but can reach 80%[6,21,22]. Pleural and pulmonary complications still account for a significant proportion of morbidity; an average of 22%[22]. Nguyen *et al*[5] have reported, in a large series of 104 patients, that postoperative major morbidity occurred in 12.5%, especially anastomotic complications, staple line leaks and pulmonary complications. Minor complications occurred in an additional 15% of cases[5].

Review papers show a median length of intensive care unit stay of 2-5 d, and a median length of hospital stay of 9-18 d after MIE[21,22]. Mortality rate ranges between 0% and 4%[5,6,20-22].

COMPARISON WITH OPEN SURGERY

As far as we are aware, no randomized controlled trials have compared MIE and open esophagectomy to date. Available data suggests that MIE is similar but not superior to conventional esophagectomy.

Morbidity and mortality

MIE was expected to reduce the morbidity and mortality rate of esophageal resection when compared to conventional surgery. However, a recent meta-analysis[23] has shown similar results for major morbidity, pulmonary complications and mortality when MIE and open surgery are compared either to transhiatal or transthoracic esophagectomy. Nguyen *et al*[5] also have shown similar pulmonary complications when MIE and open cohorts were compared. Perry *et al*[24] have compared the outcomes of open and laparoscopic transhiatal esophagectomy in two sets of patients from different periods of time. They have found that lower intraoperative blood loss and overall length of hospital stay favor MIE. Complication rates were no different.

Cost

As far as we are aware, no studies have compared cost for MIE and open surgery. It is intuitive, however, that direct operative costs are higher for MIE, especially with the use of endoscopic staplers. Moreover, the clinical benefits of MIE are not yet proven to be greatly superior to open surgery in order to decrease indirect costs.

Oncological radicality

Advantages of minimally invasive techniques include a magnified view of the operative field. This advantage theoretically enhances the ability to perform more radical lymphadenectomy. In contrast, surgeons might be less confident to work close to important vascular structures without a tactile feeling and the possibility to use their hands to control bleeding. Reported experiences with different types of cancer, such as colon[25] and stomach[26], have shown a comparable number of lymph nodes retrieved when open or minimally invasive surgery are compared. MIE shows similar results. Decker *et al*[22] have shown a mean 10-27 lymph nodes were dissected in MIE, depending on the technique adopted, and these numbers are comparable to open surgery and considered adequate[27].

Survival is expectedly similar to open surgery with an average of 40% at 5 years[22].

Learning curve

It has been shown that esophagectomy outcomes are highly linked to the experience and volume of the centers performing the operation[28]. The same seems to be true for MIE[22]. To the best of our knowledge, no studies have defined the number of procedures necessary for these techniques to become safe and effective. Advanced laparoscopic skills and experience with major foregut surgery (open and laparoscopic) are clearly necessary.

CONCLUSION

Minimally invasive surgery has the advantages of better cosmetic results, reduced operative stress, postoperative immobility, and pain. These advantages are obtained by minimizing the incisions to obtain access to natural cavities, i.e. decreasing the external surgical stress. Minimally invasive surgery does not change, however, the internal part of the operation and the surgical stress determined by it. The minimally invasive approach has gained rapid acceptance and has become the gold-standard operation where external stress is higher than internal stress, such as for cholecystectomy and hiatal hernia repair[29,30]. In operations in which internal surgical stress is intensive, such as a Whipple procedure, the minimally invasive approach is questionable[31]. This is also true for MIE. This review shows that, even with a minimally invasive approach, patients are not discharged earlier and the clinical consequences of intense internal aggression, such as systemic inflammatory response syndrome[32], are still noticed after MIE. For these reasons and for the

technical skills necessary to perform a MIE, it is not a disseminated and widely used approach for esophageal resection. Boone *et al*[33] have surveyed 269 surgeons, members of the International Society for Diseases of the Esophagus, the European Society of Esophagology Group, and the World Organization for Specialized Studies on Diseases of the Esophagus. They have found that MIE was the operation of choice for only 14% of the responders, while 60% of them never used the MIE approach. Similar results have been presented by Enestvedt *et al*[34]. Not surprisingly, they also have shown that MIE is performed more frequently by high-volume surgeons compared to those from low-volume centers.

The available literature on MIE is still crowded with heterogeneous studies with different techniques. As far as we are aware, there have been no controlled comparative trials, and the few retrospective comparative cohort studies have been limited by small numbers of patients and biased by historical controls of open surgery[22]. Moreover, few studies have included > 100 patients. Based on the available literature, there is no current evidence that MIE brings clear benefits compared to conventional esophagectomy. Growing experience and studies with larger numbers of patients could change this situation.

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ORIGINAL ARTICLE

Portal vein ligation accelerates tumor growth in ligated, but not contralateral lobes

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Abstract

AIM: To investigate the mechanisms of liver growth and atrophy after portal vein ligation (PVL) and its effects on tumor growth.

METHODS: Mice were subjected to PVL, partial hepatectomy, or sham surgery. The morphological alterations, activation of transcription factors, and expression of cytokines and growth factors involved in liver regeneration were evaluated. In a separate set of experiments, murine colorectal carcinoma cells were injected *via* the portal vein and the effect of each operation on liver tumor growth was studied.

RESULTS: Liver regeneration after PVL and partial hepatectomy were very similar. In ligated lobes, various cytokines, transcription factors and regulatory factors were significantly upregulated compared to non-ligated lobes after PVL. Atrophy in ligated lobes was a result of early necrosis followed by later apoptosis. Tumor growth was significantly accelerated in ligated compared to non-ligated lobes.

CONCLUSION: Tumor growth was accelerated in ligated liver lobes and appeared to be a result of increased growth factor expression.

Key words: Portal vein ligation; Tumor growth; Growth factor; Atrophy; Apoptosis

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INTRODUCTION

Liver resection is the standard treatment for patients with primary or secondary liver malignancies, and offers the only chance of long-term survival[1,2]. With advances in surgical techniques, perioperative management, and anatomical knowledge of the liver, major hepatectomy usually does not carry a high operative mortality in patients with normal hepatic function, or in those with large tumors, unless accompanied by technical failure. However, the morbidity and mortality after extensive hepatectomy, or major hepatectomy in patients with obstructive jaundice, hepatic dysfunction, or tumors increase due to postop-

erative liver failure caused by excessive loss of functional residual liver mass[3,4]. It has been reported that there is a strong correlation between the expected remnant liver volume and postoperative liver failure in patients who undergo liver resection[5]. Surgical resection of liver tumors requires a sufficient surgical margin that can lead to substantial loss of residual mass. However, it is essential to secure sufficient functional liver mass to prevent postoperative liver failure.

In 1920, Rous and Larimore showed that selective portal occlusion can produce atrophy of the occluded lobe and compensatory hypertrophy of the contralateral lobe in rabbits[6]. In the clinical setting, Makuuchi *et al*[7] first proposed portal vein embolization as a preoperative treatment to avoid postoperative liver failure due to insufficient remnant liver mass. Portal vein embolization is now widely accepted as a useful procedure to extend eligibility of patients with liver cancer for liver resection. However, one study has shown that some patients can become ineligible for scheduled surgery due to tumor progression after portal vein embolization[8], whereas another study has indicated that portal vein embolization neither prevents nor accelerates tumor growth[9]. Thus, the effect of portal vein embolization on tumor growth prior to resection is not well understood.

In the present study, we used a murine model of portal vein ligation (PVL) to determine the effects of ligation on the mechanisms of liver growth and regeneration. In addition, we evaluated how these mechanisms influence the growth of colorectal carcinoma tumors in ligated and contralateral lobes after PVL.

MATERIALS AND METHODS

Animal model

Male C57BL/6J and BALB/c mice (Jackson Laboratory, Bar Harbor, ME, USA) weighing 20-26 g were used in all experiments. This project was approved by the University of Cincinnati Animal Care and Use Committee and was in compliance with the National Institutes of Health guidelines. The C57BL/6J mice were randomly separated into a PVL group, partial hepatectomy group, and sham operation group. All mice were anesthetized with sodium pentobarbital (60 mg/kg, ip) and a midline laparotomy was performed. For PVL, the branch of the portal vein that fed the left and median hepatic lobes, which corresponded to 70% of the whole liver, was dissected under an operative microscope and ligated with an 8-0 PROLENE suture (Ethicon, Inc., Somerville, NJ, USA). Partial hepatectomy was performed according to the method of Higgins and Anderson[10], with slight modification. 7-0 PRONOVA sutures (Ethicon) were secured around the base of the left and median hepatic lobes, and the lobes were resected. Mice were sacrificed at the indicated time points after operation, and blood and liver samples were taken for analysis. The liver lobes to body weight ratio was determined.

Blood and tissue analysis

Blood was obtained by cardiac puncture for analysis of serum alanine aminotransferase (ALT) as an index of hepatocellular injury. Measurements of serum ALT were made using a diagnosis kit by bioassay (Wiener Laboratories, Rosario, Argentina). Liver tissues were fixed in 10% neutral-buffered formalin, processed, and embedded in paraffin for light microscopy. Sections were stained with hematoxylin and eosin (HE) for histological examination. Liver content of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-1β, hepatocyte growth factor (HGF), epidermal growth factor (EGF), transforming growth factor β1 (TGFβ1) was assessed by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA). Liver samples were weighed and immediately placed in 10 volumes (wt/vol) of a protease inhibitor cocktail that contained 10 nmol/L EDTA, 2mmol/L phenylmethylsulfonyl fluoride (PMSF), 0.1 mg/mL soybean trypsin inhibitor, 1.0 mg/mL bovine serum albumin, and 0.002% sodium azide in isotonic PBS, pH 7.0. Tissues were disrupted with a tissue homogenizer, and lysates were incubated at 4℃ for 2 h. Samples were clarified by two rounds of centrifugation at 12 500 *g* for 10 min at 4℃.

Liver neutrophil accumulation

Liver myeloperoxidase (MPO) content was assessed by methods described elsewhere[11]. Liver tissue (100 mg) was homogenized in 2 mL buffer A (3.4 mmol/L KH_2HPO_4, 16 mmol/L Na_2HPO_4, pH 7.4). After being centrifuged for 20 min at 10 000 *g*, the pellet was resuspended in 10 volumes of buffer B (43.2 mmol/L KH_2HPO_4, 6.5 mmol/L Na_2HPO_4, 10 mmol/L EDTA, 0.5% hexadecyltrimethylammonium, pH 6.0) and sonicated for 10 s. After being heated for 2 h at 60℃, the supernatant was reacted with 3,3',3,5'-tetramethylbenzidine, and the optical density was read at 655 nm.

Proliferating cell nuclear antigen staining

Immunohistochemical staining for proliferating cell nuclear antigen (PCNA) was performed on paraffin-embedded liver tissue with anti-PCNA antibody using DakoCytomation ARK kit (Dako, Copenhagen, Denmark). A three-step peroxidase method was performed according to the manufacturer's instructions. PC-10 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used at a dilution of 1:50, for 15 min at room temperature. The sections were counterstained with hematoxylin. Evaluation of PC-10 immunostaining was performed based on the percentage of positive nuclei of 400-600 hepatocytes from the 4-6 highest positive fields at high power (400 ×), and was expressed as PCNA labeling index.

Western blotting

Liver samples were homogenized in lysis buffer (10 mmol/L HEPES, pH 7.9, 150 mmol/L NaCl, 1 mmol/L EDTA, 0.6% NP-40, 0.5 mmol/L PMSF, 1 μg/mL leupeptin, 1 μg/mL aprotinin, 10 μg/mL soybean trypsin inhibitor, and 1 μg/mL pepstatin). Samples were then sonicated and incubated for 30 min on ice. Cellular debris was removed by centrifugation at 10 000 r/min. Protein concentrations

of each sample were determined. Samples that contained equal amounts of protein in equal volumes of sample buffer were separated in a denaturing 10% polyacrylamide gel and transferred to a 0.1-μm pore nitrocellulose membrane. Nonspecific binding sites were blocked with Tris-buffered saline (TBS; 40 mmol/L Tris, pH 7.6, 300 mmol/L NaCl) that contained 5% non-fat dry milk for 1 h at room temperature. Membranes were then incubated with antibodies to cyclin D1 (Santa Cruz Biotechnology), signal transducer and activator of transcription 3 (STAT3) (Cell Signaling Technology, Boston, MA, USA), and phosphorylated STAT3 (Cell Signaling Technology) in TBS with 0.1% Tween 20. Membranes were washed and incubated with secondary antibodies conjugated to horseradish peroxidase. Immunoreactive proteins were detected by enhanced chemiluminescence.

Electrophoretic mobility shift assay

Nuclear extracts of liver tissue were prepared by the method of Deryckere and Gannon[12], and analyzed by electrophoretic mobility shift assay. Double-stranded consensus oligonucleotides to nuclear factor (NF)-κB and activator protein (AP)-1 (Promega, Madison, WI, USA) were end-labeled with γ[^{32}P]-ATP (3000 Ci/mmol at 10 mCi/mL; Perkin Elmer, Waltham, MA, USA). Binding reactions (total volume 15 μL) that contained equal amounts of nuclear protein extract (20 μg) and 35 fmol (approximate 50 000 cpm, Cherenkov counting) of oligonucleotide were incubated at room temperature for 30 min. Binding reaction products were separated on a 4% polyacrylamide gel and analyzed by autoradiography.

Liver tumor model

The CT26 cell line is from an undifferentiated colon adenocarcinoma induced by N-nitroso-N-methylurethane injection in BALB/c mice. The CT26.WT cell line was obtained from American Type Culture Collection (ATCC; Rockville, MD, USA). CT26.WT cells were maintained in RPMI-1640 medium (ATCC) supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin. Cells were incubated at 37℃ in a humidified atmosphere that contained 5% CO_2 in air. The cells were harvested from subconfluent cultures by 0.05% trypsinization and washed twice in PBS on the day of implantation. For the portal vein injection model, a midline incision was made and the portal vein was exposed by removing the intestine. A suspension of 2×10^5 CT26.WT cells was injected into the portal vein using a 31 G needle. After injection, a small piece of Gelfoam (Pharmacia Co., Kalamazoo, MI, USA) was pressed over the injection site for 2-3 min to obtain hemostasis. One week after tumor cell implantation, mice were subjected to portal vein ligation or sham surgery. One week after the operation, all mice were sacrificed and blood and liver samples were collected. The tumor growth was evaluated on HE slides and tumor area was determined by morphometry. Morphometric analysis was performed by image analysis software in five representative fields at low power (10 ×).

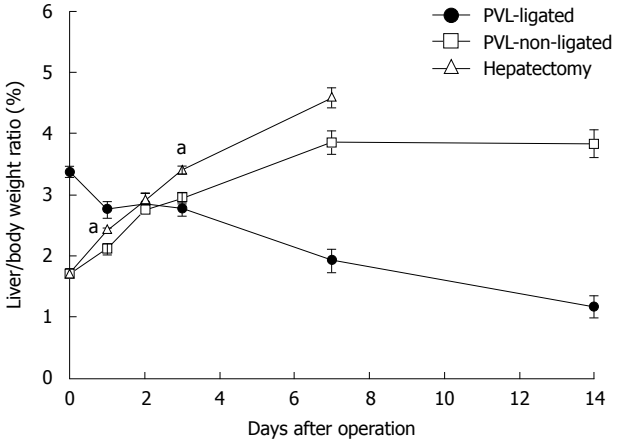

Figure 1 Changes in liver lobe to body weight ratio after portal vein ligation or partial hepatectomy. To evaluate liver regeneration after portal vein ligation (PVL) or partial hepatectomy, liver lobe to body weight ratio was determined. Data are mean ± SE with n = 4-6 per group. $^aP < 0.05$ *vs* PVL-non-ligated.

Statistical analysis

All data are expressed as mean ± SE. Data were analyzed with one-way analysis of variance with subsequent Student-Newman-Keuls test. Differences were considered significant when $P < 0.05$.

RESULTS

Liver growth and regeneration after PVL vs partial hepatectomy

To evaluate liver growth and regeneration after PVL or partial hepatectomy, we measured liver/body weight ratios. After partial hepatectomy, liver regenerated at the expected rate and returned to normal liver mass within 7 d (Figure 1). After PVL, non-ligated lobes grew at a rate similar to liver after partial hepatectomy, but reached a plateau of mass below that after partial hepatectomy (Figure 1). The ligated lobes atrophied at a constant rate and after 14 d, the mass of the ligated lobes was approximately one third of the starting mass (Figure 1).

In accordance with the changes in liver growth and regeneration, similar patterns were found when we examined hepatocyte proliferation by staining for PCNA. PCNA-positive hepatocytes increased in a similar fashion after partial hepatectomy and in non-ligated lobes after PVL (Figure 2). However, there were subtle differences noted. While the number of PCNA-positive hepatocytes was maximal in both partial hepatectomy and non-ligated lobes after PVL at 2 d after surgery, there were significantly more PCNA-positive hepatocytes in the partial hepatectomy group (Figure 2). Furthermore, the number of PCNA-positive hepatocytes dropped dramatically by day 3 after partial hepatectomy, whereas after PVL, the number of PCNA-positive hepatocytes in non-ligated lobes had a more gradual decrease and was significantly higher compared to that after partial hepatectomy (Figure 2). Despite these minor differences, our data confirm previous studies that the mechanisms of liver growth and regeneration are

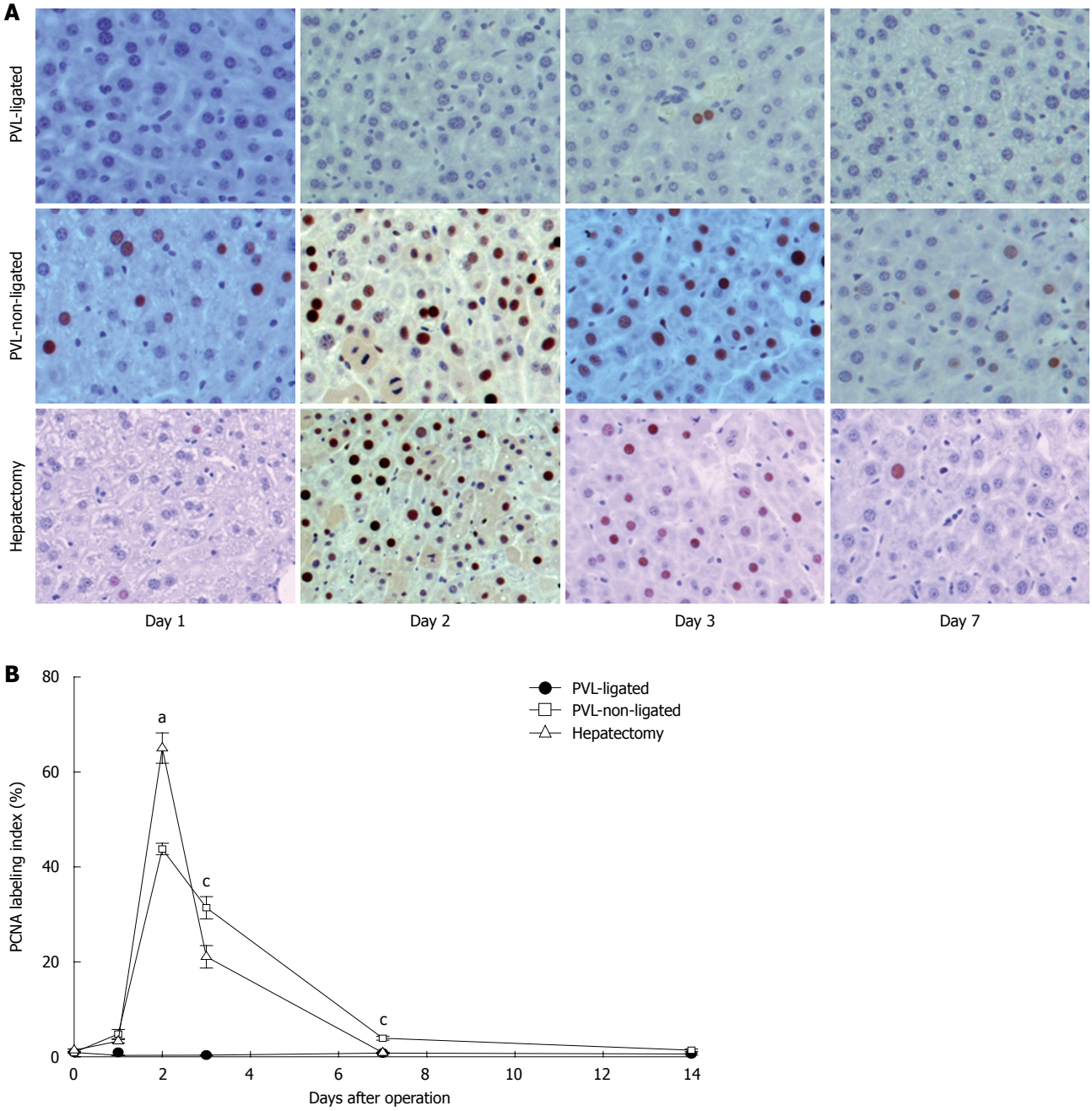

Figure 2 Hepatocyte proliferation after portal vein ligation. A: Hepatocyte proliferation was determined by immunohistochemical staining for proliferating cell nuclear antigen (PCNA). Original magnification was 200 ×; B: Quantitative analysis of PCNA labeling. PCNA labeling index was expressed as percentage of positive nuclei of 400-600 hepatocytes from the 4-6 highest positive fields at high power (400 ×). Data are mean ± SE with n = 4-6 per group. $^{a}P < 0.05$ *vs* PVL-non-ligated; $^{c}P < 0.05$ *vs* partial hepatectomy.

similar between that occurring in non-ligated lobes after PVL and that occurring after partial hepatectomy[13].

We next examined the mode of cell death after PVL. Serum levels of ALT were assessed as a measure of hepatocyte necrosis and TUNEL staining was performed to determine the amount of hepatocyte apoptosis. Serum levels of ALT peaked 1 d after PVL, but remained elevated for 14 d (Figure 3A). Corresponding with the ALT data, ligated lobes showed areas of necrosis within 1 d after PVL (Figure 3B, upper panels). These regions persisted for up to 7 d after PVL and were undetectable by day 14. In contrast, significant hepatocyte apoptosis was detected in ligated lobes, beginning at 7 d after PVL and persisting until 14 d after PVL (Figure 3B, lower panels). In non-ligated lobes, no evidence of hepatocyte necrosis or apoptosis was observed (data not shown).

Lobar differences in cytokine and growth factor expression after PVL

A variety of cytokines and growth factors are known to modulate liver growth and regeneration. To evaluate whether expression of relevant cytokines and growth factors is related to the growth of non-ligated lobes and/or the atrophy of ligated lobes, we measured the protein

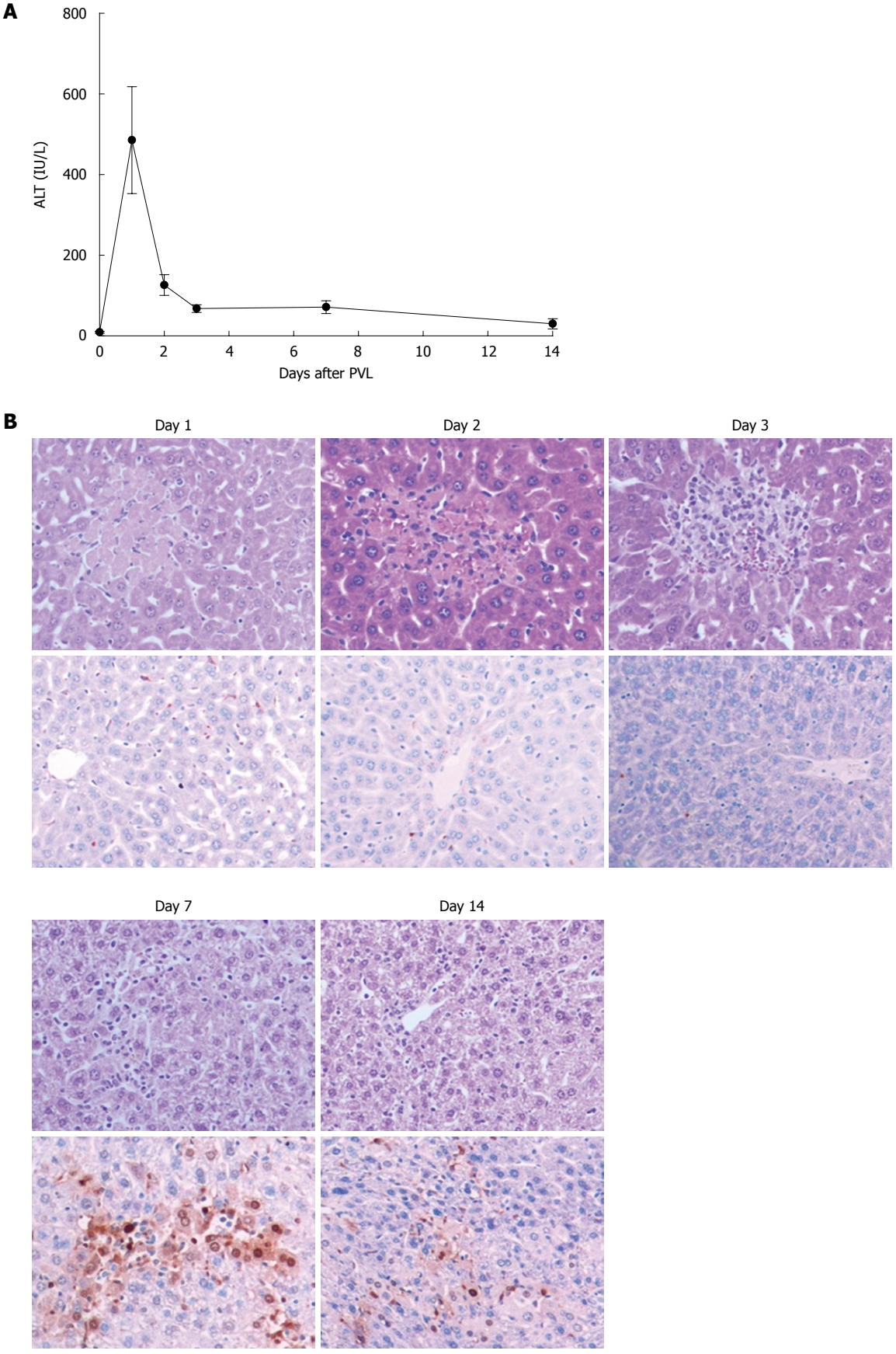

Figure 3 Effects of portal vein ligation on liver necrosis and apoptosis. A: Liver injury was measured by serum levels of alanine aminotransferase (ALT). Data are mean ± SE with n = 4-6 per group; B: Representative pictures of HE staining (upper) and TUNEL staining (bottom). TUNEL staining was performed to determine the amount of hepatocyte apoptosis. Original magnification was 200 ×. PVL: Portal vein ligation.

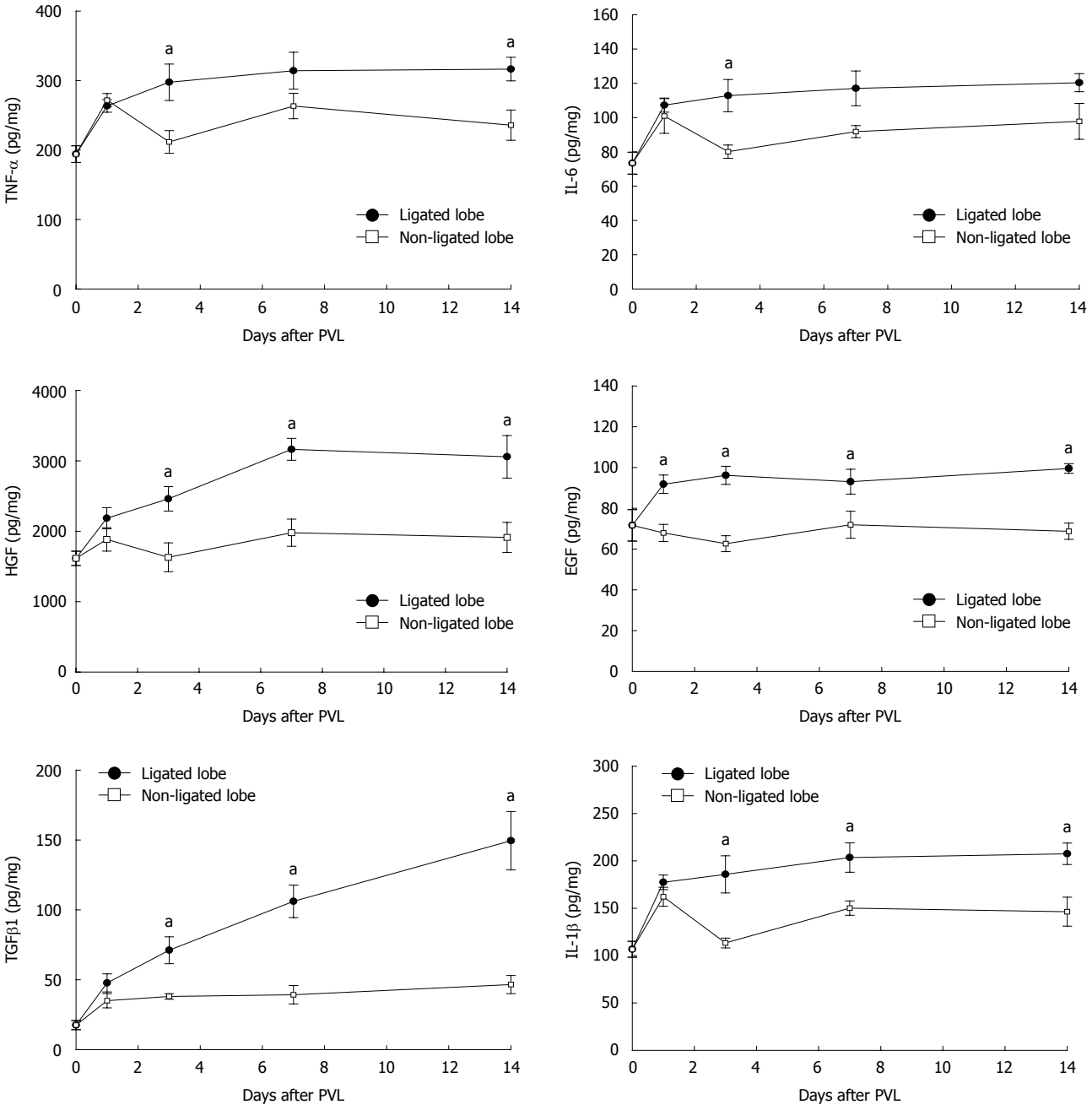

Figure 4 Effect of portal vein ligation on liver cytokines, growth factors, and chemokines. To evaluate whether expression of relevant cytokines and growth factors are related to the growth of non-ligated lobes and/or the atrophy of ligated lobes, liver levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, hepatocyte growth factor (HGF), epidermal growth factor (EGF), transforming growth factor β1 (TGFβ1), and IL-1β were analyzed by enzyme-linked immunosorbent assay (ELISA). Liver lysates were processed for ELISA. Data are mean ± SE with n = 4-15 per group. $^{a}P < 0.05$ *vs* portal vein ligation (PVL)-non-ligated.

levels of HGF, EGF, TNF-α, IL-6, TGFβ1, and IL-1β in liver tissues. HGF and EGF are direct mitogens for hepatocytes and are crucial inducers of liver regeneration[14-16]. Expression of HGF and EGF were increased in both ligated and non-ligated lobes after PVL (Figure 4). However, expression of these mediators was much higher in ligated lobes compared to non-ligated lobes. TNF-α and IL-6 have been implicated as important contributors to liver growth and regeneration[14-16]. Expression of TNF-α and IL-6 increased similarly at 1 d after PVL in ligated and non-ligated lobes (Figure 4). By day 3, expression of TNF-α and IL-6 was significantly higher in ligated lobes compared to non-ligated lobes. TGFβ1 and IL-1β are known as suppressors of cell proliferation and might be involved in termination of liver regeneration[15,17]. We found that expression of TGFβ1 and IL-1β was increased 1 d after PVL in both ligated and non-ligated lobes (Figure 4). However, by day 3, expression of TGFβ1 had reached a plateau and IL-1β decreased in non-ligated lobes, whereas their expression had increased further in ligated lobes.

Divergent signaling mechanisms in ligated and non-ligated lobes after PVL

NF-κB, AP-1 and STAT3 are known to be important mediators of liver growth and regeneration[18,19], therefore,

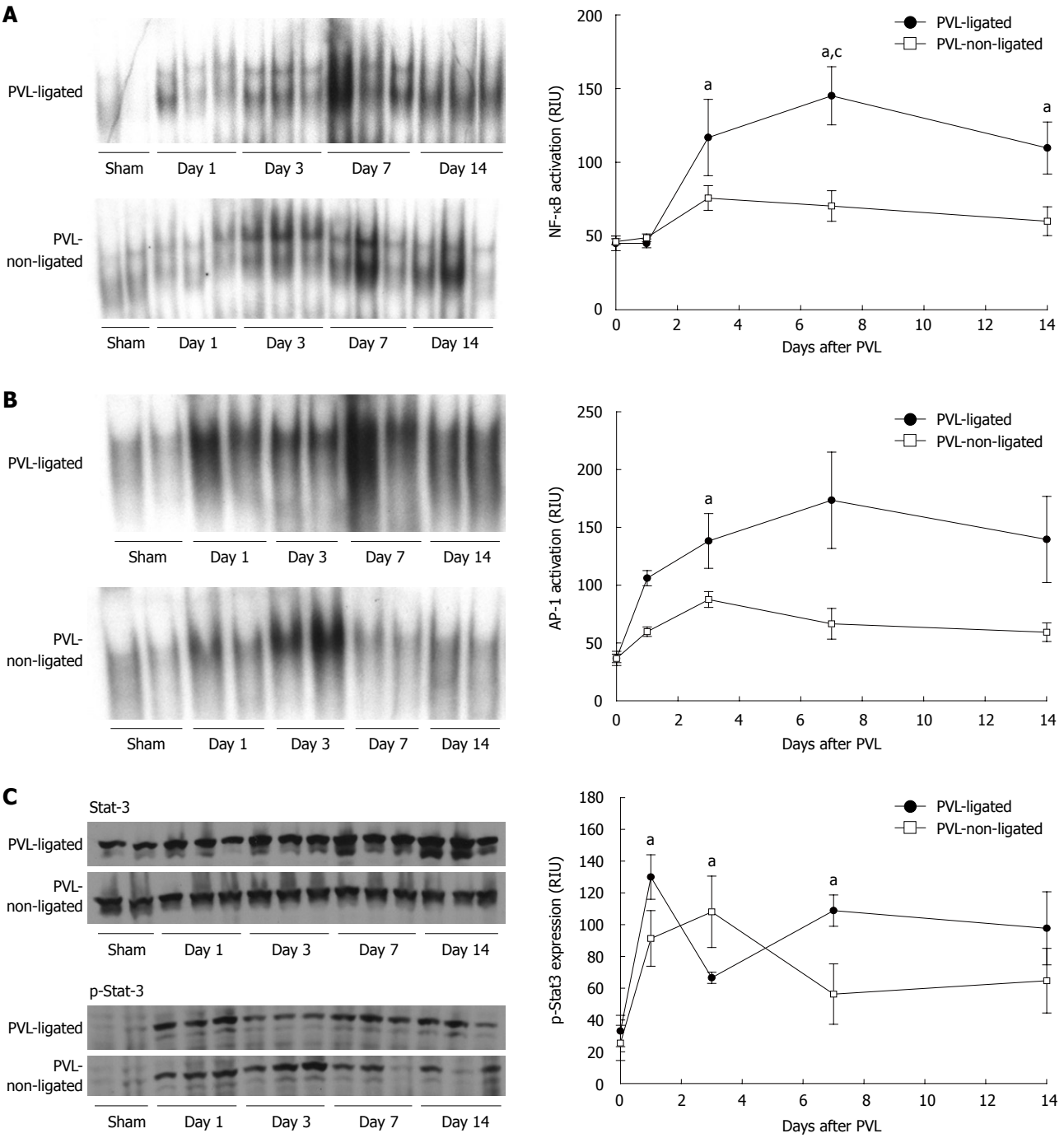

Figure 5 Transcription factor activation after portal vein ligation. Nuclear factor (NF)-κB (A), activator protein (AP)-1 (B) and signal transducer and activator of transcription 3 (STAT3) (C) were examined in liver extracts. For NF-κB and AP-1, liver nuclear extracts were analyzed by electrophoretic mobility shift assay. For STAT3, liver lysates were assessed by Western blotting. Results were quantitated by image analysis of autoradiograms and chemiluminescence films. Data are mean ± SE with $n = 4$ per group. A: $^{a}P < 0.05$ *vs* sham-operated group; $^{c}P < 0.05$ *vs* portal vein ligation (PVL)-non-ligated group; B: $^{a}P < 0.05$ *vs* PVL-non-ligated group; C: $^{a}P < 0.05$ *vs* sham-operated group.

we assessed the activation of these transcription factors in ligated and non-ligated lobes after PVL. NF-κB activation increased in both ligated and non-ligated lobes by day 3 after PVL; however, it was much greater in ligated lobes (Figure 5A). In non-ligated lobes, NF-κB activation remained elevated, albeit modestly, throughout the 14-d experimental period (Figure 5A). In contrast, activation of NF-κB in ligated lobes increased further, and remained significantly higher than in non-ligated lobes (Figure 5A). Supershift assays of NF-κB from each lobe indicated that the composition was composed primarily of p50/p65 heterodimers (data not shown).

In contrast to NF-κB, which did not become activated until 3 d after surgery, AP-1 activation occurred rapidly after PVL in both ligated and non-ligated lobes (Figure 5B). In non-ligated lobes, activation of AP-1 was increased modestly throughout the 14-d experiment. Similar to NF-κB, activation of AP-1 was much greater in the ligated

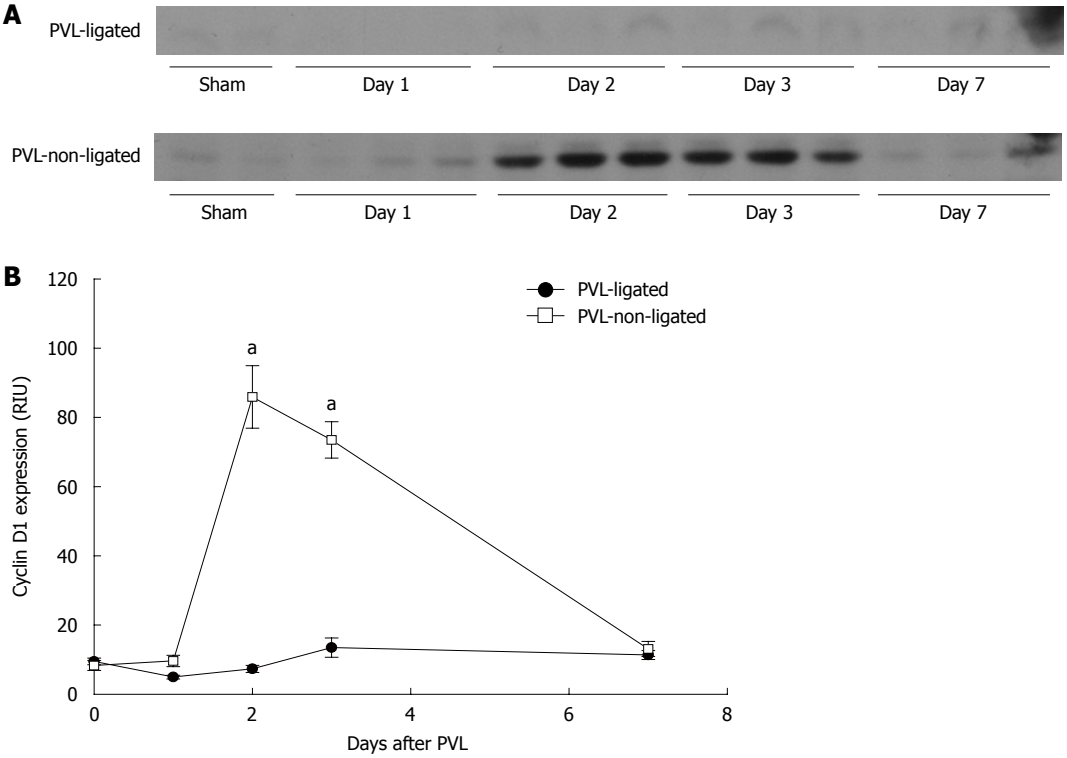

Figure 6 Liver cyclin D1 expression after portal vein ligation. A: Liver lysates were assessed for cyclin D1 protein expression by Western blotting; B: Chemiluminescence films were quantitated by image analysis. Data are mean ± SE with $n = 4$ per group. $^{a}P < 0.05$ *vs* portal vein ligation (PVL)-ligated group.

lobes compared to non-ligated lobes at every time point (Figure 5B). Supershift assays determined that the composition of AP-1 was similar in each lobe, primarily c-Fos, JunB, and JunD (data not shown).

STAT3 activation, as determined by STAT3 phosphorylation, was rapidly increased in both ligated and non-ligated lobes after PVL (Figure 5C). Interestingly, STAT3 activation decreased in ligated lobes at day 3 and then increased at days 7 and 14. In contrast, STAT3 activation in non-ligated lobes peaked at day 3 and then decreased at days 7 and 14.

Cyclin D1 is known to play a crucial role in the control of hepatocyte proliferation from G1- to S-phase[16,20,21]. Expression of cyclin D1 in non-ligated lobes was significantly increased after PVL, whereas there was no induction of cyclin D1 expression in ligated lobes (Figure 6).

PVL accelerates tumor growth in ligated, but not in non-ligated lobes

To investigate how the different milieus in ligated *vs* non-ligated lobes might alter the growth of liver tumors, mice were injected *via* the portal vein with murine colorectal carcinoma cells 7 d prior to PVL or sham surgery. We used murine colorectal carcinoma cells, CT26.WT, to reproduce the nature of colorectal liver metastases by injecting the cells into the portal vein. In sham-operated mice, there were similar amounts of small tumors in lobes that corresponded to ligated and non-ligated lobes (Figure 7A and B). In mice undergoing PVL, ligated lobes had large tumor nodules that were clearly visible on gross examination as well as histologically (Figure 7A and B). Quantitation of tumor area in liver sections demonstrated a four-fold increase in relative tumor size in ligated lobes *vs* non-ligated lobes (Figure 7C).

DISCUSSION

In the current study, we evaluated the effects of PVL on expression of cytokines and growth factors and signaling pathways that are known to contribute to liver growth and regeneration. Although the trigger for growth of contralateral lobes after PVL has not been fully elucidated, hemodynamic changes after PVL have been proposed as an initial event that contributes to this process[22]. Following PVL, arterial blood flow to the ligated lobe roughly doubles, while arterial blood flow to the non-ligated lobes is roughly 60% of normal[22]. Portal flow to the non-ligated lobes, however, more than doubles[22] and this increase in supply helps trigger growth mechanisms in the non-ligated lobes[23]. Hemodynamic changes are more drastic after partial hepatectomy because both arterial and portal flows to the remnant liver are increased. The difference in hemodynamic changes between PVL and partial hepatectomy could affect the degree and/or timing of expression of some proteins involved in regeneration[13,24] and cause a slight delay of regeneration in the non-ligated lobes. However, the gross regenerative responses are similar, as shown by our data.

Some studies have shown that the early growth/regenerative response, including activation of NF-κB, STAT3, IL-6, c-fos, c-myc, and c-jun, are similarly induced in both ligated and non-ligated lobes[18]. Other studies have shown

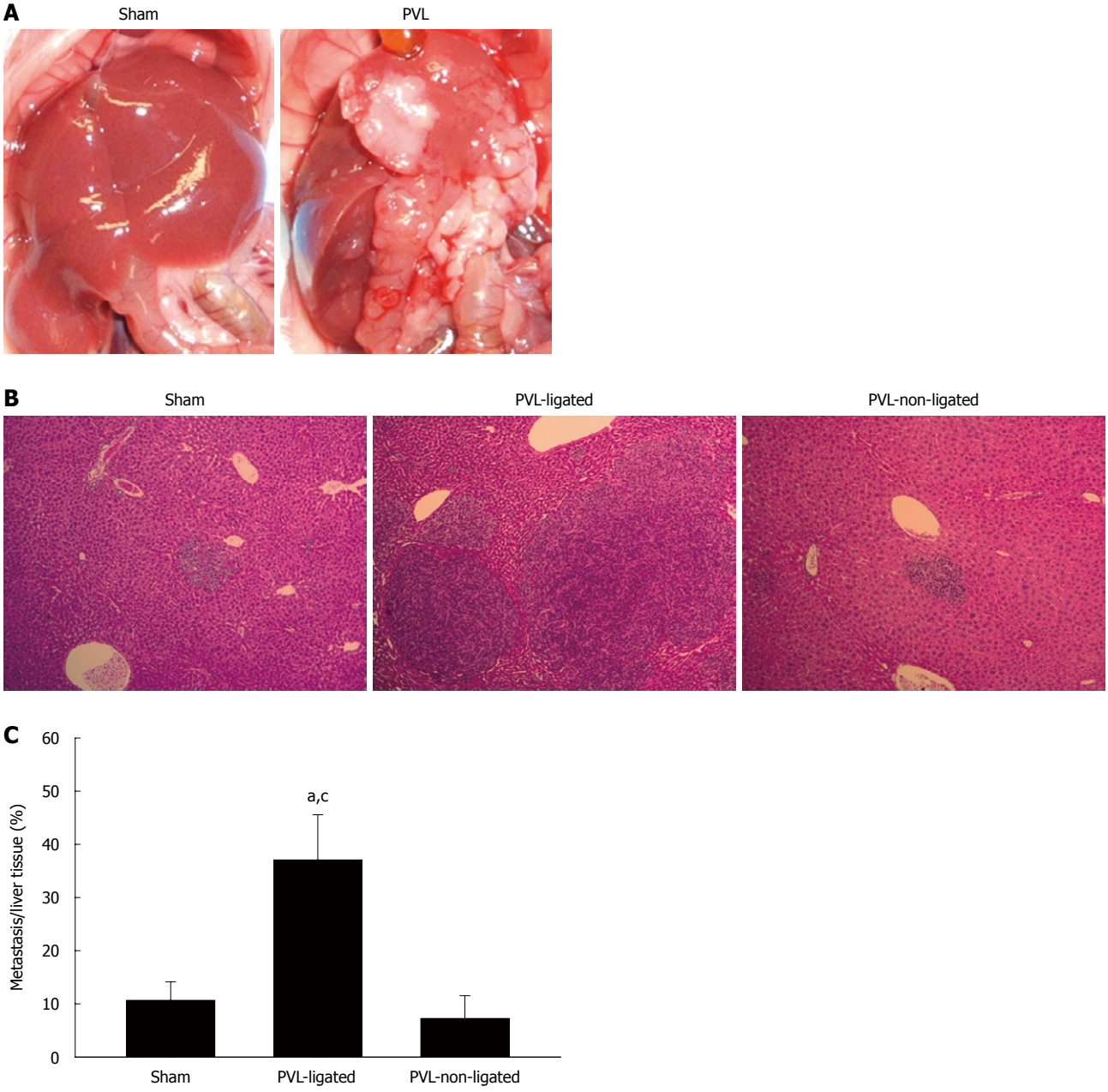

Figure 7 Effect of portal vein ligation on tumor growth. A: CT26.WT cells were injected into portal the vein. Mice were sacrificed at 14 d after injection following sham operation or portal vein ligation (PVL) performed on day 7 after injection; B: Representative pictures of liver histology after PVL. Small metastatic foci were observed in sham-operated and non-ligated lobes. Large metastatic foci were observed in ligated lobes. Original magnification was 10 ×; C: The ratio of metastases to normal liver was measured by morphometry. Data are mean ± SE with $n = 4$ per group. $^{a}P < 0.05$ *vs* sham-operated group; $^{c}P < 0.05$ *vs* PVL-non-ligated group.

differences in growth factor mRNA expression between ligated and non-ligated lobes[25]. Our data demonstrate that these growth and regenerative mechanisms are greatly increased in both ligated and non-ligated lobes, but are significantly greater in the ligated compared to non-ligated lobes. Cyclin D1 is the sole exception, being induced only in the non-ligated lobes. Cyclin D1 is known to play a crucial role in the control of hepatocyte proliferation from G1- to S-phase[20,21], and appears to be the determinant of proliferation or atrophy in non-ligated and ligated lobes, respectively.

The milieu in the ligated lobe, with greatly increased expression of cytokines and growth factors and increased activation of NF-κB and AP-1, is rather chaotic and not indicative of either a "survival" or "death" mode. TNF-α can function to promote hepatocyte proliferation or death, depending on the co-stimuli present[26-28]. NF-κB activation in hepatocytes is pro-survival and anti-apoptotic[29,30], whereas activation of AP-1 promotes hepatocellular injury and apoptosis[31]. The fate of the ligated lobe might be less dependent upon the changes in these factors, and more on the lack of nutrient and oxygen delivery. As is clear, the end result is atrophy of the ligated lobe through necrotic and apoptotic mechanisms. Despite this atrophy and the pro-hepatocyte death milieu, colorectal carcinoma metastases grew much faster in the ligated lobes compared to the non-ligated lobes after PVL. These findings are consistent with other studies

that have shown increased tumor growth in the ligated lobes after PVL[32,33]. However, our study offers more insight into the potential mechanisms that contribute to the increased tumor growth, as our data provide important information about the expression of growth factors and signaling pathways. HGF and EGF have a stimulatory effect on tumor cells[34,35], and therefore, the increased HGF and EGF observed in the ligated lobe after PVL could explain the accelerated tumor growth. TGFβ1 was also increased in the ligated lobe. Although TGFβ1 is generally known as a negative regulator in liver regeneration[15], some recent studies have reported a tumor promoter role for TGFβ1 in hepatocellular carcinoma and liver metastasis[36-38]. It has been shown that TGFβ1 is highly proliferative in CT26 cells, the colorectal carcinoma cell line used in our studies[39]. Furthermore, TGFβ1 is known to contribute to hepatocyte apoptosis, and colorectal carcinoma cells secrete significant amounts of TGFβ1, which might contribute to tumor growth[39,40]. Therefore, it is plausible that increased TGFβ1 expression in the ligated lobes significantly contributed to the accelerated growth of colorectal carcinoma tumors after PVL.

In summary, the present study demonstrated the signaling pathways that were activated in ligated and non-ligated lobes after PVL. Both lobes had increased expression of pro-proliferative cytokines and growth factors, as well as activation of pro-regenerative transcription factors, which help to define the molecular events that contribute to growth of contralateral lobes. Ligated liver lobes had significant increases in proliferative cytokines, growth factors and transcription factors compared to non-ligated lobes. While this response might constitute a survival mode for the hepatic parenchyma, it appears to provide an environment that facilitates tumor growth. PVL is a proven modality for increasing the functional liver remnant and extending the indications for surgery for metastatic liver disease. Future studies are needed to assess the effects of adjuvant or neoadjuvant chemotherapy on the hepatic expression of growth factors and tumor growth rate in this model.

COMMENTS

Background

Liver resection is the standard treatment for patients with primary or secondary liver malignancies, and offers the only chance of long-term survival. Although, the outcome of hepatic resection is improving, postoperative liver failure that results from insufficient functional liver volume after surgery could be lethal. Portal vein embolization is now widely accepted as a useful procedure to increase remnant liver volume and extend eligibility of patients with liver cancer for liver resection.

Research frontiers

Portal vein embolization is well known to induce hypertrophy of contralateral lobes. However, the manner in which portal vein embolization alters growth of the contralateral lobes and atrophy of the embolized lobe(s) is incompletely understood. Moreover, the effect of portal vein embolization on tumor growth is controversial.

Innovations and breakthroughs

In the current study, the authors demonstrated that various cytokines, transcription factors and regulatory factors were significantly upregulated in ligated lobes compared to non-ligated lobes after portal vein ligation. Tumor growth was accelerated in the ligated compared to non-ligated lobes and appeared to be a result of increased growth factor expression.

Applications

The results provide strong evidence of accelerated tumor growth in ligated lobes. This should be taken into account for the treatment strategy when patients undergo portal vein embolization.

Peer review

The experiments were well designed and well conducted. The topic relates to the advantages and/or disadvantages of the surgical procedure of portal vein embolization prior to major liver resections for hepatocellular carcinoma or other liver cancers.

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ORIGINAL ARTICLE

Decreased IgA+ plasma cells and IgA expression in acute liver necrosis mice

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Abstract

AIM: To investigate the number of intestinal immunoglobulin A (IgA+) plasma cells and expression of intestinal IgA in mice with acute liver necrosis.

METHODS: A model of acute liver necrosis was established by intraperitoneal injection of galactosamine (GalN) and lipopolysaccharide (LPS). Sixty mice were randomly divided into one of 4 equal groups: normal control, acute liver necrosis, LPS, or GalN. Hematoxylin and eosin staining, immunohistochemistry, and an enzyme-linked immunosorbent assay were employed to assess liver and intestinal injury, count intestinal IgA+ plasma cells, and measure the expression level of IgA and interferon γ (IFN-γ) in the small intestinal mucosa of mice.

RESULTS: Injured intestinal mucosa was observed in the acute liver necrosis group but not in the normal, LPS or GalN groups. Compared with the normal group, intestinal IgA+ plasma cells were slightly decreased in the LPS and GalN groups [429 ± 20 per high power field (HPF), 406 ± 18/HPF, respectively], whereas they were markedly decreased in the acute liver necrosis group (282 ± 17/HPF *vs* 495 ± 26/HPF in normal group, $P < 0.05$). The expression of intestinal IgA was also slightly decreased in LPS and GalN groups, but was markedly reduced in the acute liver necrosis group as determined by enzyme-linked immunosorbent assay ($P < 0.05$). In contrast, the level of IFN-γ was slightly increased in LPS, GalN and acute liver necrosis groups, but with no statistical significance ($P > 0.05$).

CONCLUSION: Intestinal IgA+ plasma cells and IgA expression levels indicating that mucosal immune barrier dysfunction, does exist in acute liver necrosis.

Key words: Acute liver necrosis; Intestinal mucosa; Immunity; Immunoglobulin A

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Fu JL, Wang ZH, Li GZ, Wang YR, Liu P. Decreased IgA+ plasma cells and IgA expression in acute liver necrosis mice. *World J Gastroenterol* 2010; 16(30): 3827-3833 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i30/3827.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i30.3827

INTRODUCTION

Patients with acute liver necrosis are at high risk for enterogenic infections. Enterogenic infections are an important cause of death in patients with acute liver

necrosis associated with intestinal barrier injury, including immunological barrier injury[1,2]. Immunoglobulin A (IgA) is an important component of the intestinal immunological barrier and is the most abundant immunoglobulin at mucosal surfaces where it plays crucial roles in mucosal protection[3]. The protective barrier of the gastrointestinal system is impaired in IgA deficiencies, and IgA-deficient individuals have a tendency to develop gastrointestinal infections[4]. Previous studies have shown decreased levels of secretory IgA and decreased numbers of IgA+ plasma cells in the intestinal tract during stress and thermal injury suggesting that the humoral immune function was dramatically inhibited in these situations[5,6]. Intestinal IgA was also decreased in endotoxemia and intra-abdominal sepsis models[7,8].

Previous studies have primarily focused on mechanical barrier interruption in acute liver necrosis models[9]. So far, no studies have shown a role for the intestinal immunological barrier in acute liver necrosis. It has been reported that an increase in levels of interferon γ (IFN-γ), a TH1 cytokine, was related to tissue injury[10] and led to a decreased expression of IgA[11].

This study set out to determine whether the number of intestinal IgA+ plasma cells and the expression of IgA were modified in mice with acute liver necrosis, in an attempt to establish whether dysfunction of the intestinal immunological barrier occurs during acute liver necrosis. In addition, IFN-γ levels in the intestinal mucosa were also evaluated.

MATERIALS AND METHODS

Animals

Sixty male BALB/c mice 6-8 wk of age (provided by the Laboratory Animal Center of the China Medical University) were housed under constant room temperature and humidity, and subjected to a 12 h light/dark cycle. Experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals. Mice were equally and randomly divided into one of 4 groups: normal control, acute liver necrosis, lipopolysaccharide (LPS), or galactosamine (GalN). GalN (800 mg/kg body weight, Sigma, USA) and LPS (10 μg/kg body weight, Sigma, USA) were injected intraperitoneally to induce acute liver necrosis as previously described[12,13]. Serum, liver and proximal small intestinal tissues samples were obtained 9 h after GalN/LPS injection.

Blood biochemistry assay

Serum alanine transaminase (ALT) levels were determined using an automatic analyzer (Hitachi 7250; Hitachi, Japan).

Histological testing

The liver and proximal small intestinal tissue were separately stored in formalin, and embedded by paraffin. The liver and intestinal sections were cut at a thickness of 5 μm and stained with hematoxylin and eosin (HE) to explore the histopathological changes in the liver and intestinal mucosa.

Immunohistochemistry for intestinal IgA+ plasma cells

Intestinal IgA+ plasma cells were investigated by immunohistochemistry (IHC). Sections of proximal small intestine were deparaffined, and antigen retrieval was performed by pressure cooker boiling for 2 min in 10 mmol/L citrate buffer (pH 6.0). IHC analysis was performed using goat anti-mouse IgA (Zymed, USA, diluted 1:50) for 12 h at 4℃, and the secondary antibody (rabbit anti-goat IgG) was applied for 2 h at 37℃. Fresh peroxidase reaction mixture containing equal amounts of 0.02% hydrogen peroxide in H_2O and 0.1% diaminobenzidine in H_2O were prepared. Sections were mounted on Uvinert mountant (BDH, UK). Five fields of small intestinal mucosa lamina propria were examined in each section at high magnification (200 ×), and the number of IgA+ cells were counted (i.e. lymphocytes that stained a brownish-yellow color). The average number of IgA+ was calculated.

Enzyme-linked immunosorbent assay for IgA expression

The levels of intestinal IgA were examined by enzyme-linked immunosorbent assay (ELISA). Intestinal tissue (50 mol/Lg) immersed in 1 mL (10 volumes, w/v) of phosphate-buffered saline (PBS) was incubated at room temperature for 15 min. Samples were vortexed, left to settle for 15 min, revortexed until all material was suspended, then centrifuged at 12000 r/min for 10 min. The supernatant was collected and tested on an ELISA kit for IgA (Bethyl Laboratories, Montgomery, TX, USA). Briefly, 96-well microtiter plates were coated with goat anti-mouse IgA affinity purified antibody and incubated for 60 min, then washed with PBS, and each well was incubated with 1% bovine serum albumin in PBS to block any nonspecific binding. After washing with PBS containing 0.1% Tween-20, 100 μL test samples and 100 mol/L standards were added into each well and incubated for 60 min followed by incubation with peroxidase-labeled goat anti-mouse specific IgA antibody for 30 min. Then 0.1 mol/L acetate buffer containing 1 mg/mL ortho-phenylenediamine was prepared and 3 μL of the prepared solution in 10 mL of H_2O_2 was added to each well. The reactions were stopped by adding 25 μL of 2 mol/L sulfuric acid. The absorbance of each solution was determined at a wavelength of 450 nm.

Detection of IFN-γ in the intestinal mucosa

The small intestinal mucosa homogenate was prepared as described previously[8]. The levels of IFN-γ in the homogenate were measured by sandwich ELISA (Quantikine ELISA Kits, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Statistical analysis

Software SPSS 11.0 was used for statistical analysis. Each value was expressed as the mean ± SE, and compared by using one-way ANOVA, followed by the Tukey test. $P < 0.05$ was considered statistically significant.

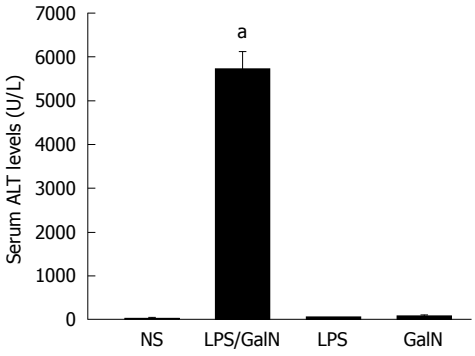

Figure 1 Serum alanine transaminase levels. Each value was expressed as mean ± SE. [a]$P < 0.05$ *vs* normal saline (NS). ALT: Alanine transaminase; GalN: Galactosamine; LPS: Lipopolysaccharide.

RESULTS

Mortality rate of mice and serum ALT levels

In the acute liver necrosis group, the mortality rate was 53.3% (8/15), compared to 0% in the other control groups. The serum ALT levels in LPS, GalN and normal groups were almost at the same level (44.3 ± 12.1, 74.2 ± 14.3, and 24.8 ± 14.7 U/L, respectively), but increased significantly in the acute liver necrosis group (5730.1 ± 383.5 U/L *vs* 24.8 ± 14.7 U/L, $P < 0.05$) (Figure 1).

Assessment of liver and proximal small intestinal injury with HE staining

In the normal group, the liver clearly showed normal structure of both the hepatic lobuli and hepatic cords. In contrast, the livers from the acute necrosis group had severe hemorrhage, hepatic necrosis, acidophilic degeneration in some residual hepatocytes, disappearance of hepatic cords, and deranged structure of hepatic lobules. Acidophilic degeneration and swelling were observed in the LPS group and edematous and spotty necrosis, as well as a few hepatic cells with acidophilic changes were found in the GalN group (Figure 2).

In normal, LPS and GalN groups, the intestinal mucosa was complete and the intestinal cells appeared ordered. In contrast, the intestinal mucosa of mice with acute liver necrosis were loosened and some of epithelial cells were edematous and necrotic (Figure 3).

IHC for IgA+ plasma cells

As shown in Figure 4, the number of IgA+ plasma cells within the lamina propria (as determined by IHC) were 495 ± 26/high power field (HPF), 282 ± 17/HPF, 429 ± 20/HPF and 406 ± 18/HPF in the normal, acute liver necrosis, LPS and GalN groups, respectively. The LPS group and GalN group had slightly lower numbers of IgA+ plasma cells than the normal group; however, the acute liver necrosis group had significantly lower numbers of IgA+ plasma cells compared to the other groups ($P < 0.05$, Figure 5A).

ELISA measurement of IgA

There was a slight decrease in the expression of IgA in LPS and GalN groups whereas no difference was noted in IgA expression in the small intestine compared with normal control. IgA expression in the small intestines from mice in the acute liver necrosis group was markedly reduced compared with the normal group ($P < 0.05$, Figure 5B).

IFN-γ in small intestinal mucosa

There were a slight increase in IFN-γ levels in LPS, GalN and acute liver necrosis groups, but no significant difference was noted compared with the normal group ($P > 0.05$, Figure 5C).

DISCUSSION

Acute liver necrosis is associated with a high mortality rate[14]. Infection is a common serious complication of acute liver necrosis and is a major cause of death[15]. A myriad of researchers have noted that secondary infections primarily originate from intestinal bacterial translocation. While the intestinal barrier has to be permeable for nutrients and macromolecules which are indispensable for growth and development, at the same time it also has to provide an effective barrier against harmful macromolecules and microorganisms to ensure local homeostasis[16]. The intestinal barrier consists of a mechanical barrier, immunological barrier, microorganism barrier, and a chemical barrier. The immunological barrier is considered the first line of antigen-specific immune defense against pathogenic microorganisms[17,18]. Recent reports indicated that immunosuppression, involving the local intestinal immunological barrier, is a major cause of intestinal bacterial translocation[19].

In agreement with previous reports[20,21], we found that injection of GalN/LPS induced increases in serum ALT and the development of severe hepatocyte necrosis. As the mortality of mice with acute liver necrosis was 53.3%, these findings indicate that the model employed to study IgA and IFN-γ was successful. In addition to the observed liver injury, loosened intestinal mucosa and some edematous and necrotic intestinal epithelial cells were also noted in the mice with acute liver necrosis. These features were not found in any of the other groups. These histological findings of intestinal mucosal injury in acute liver necrosis were consistent with other studies[14].

IgA is the most abundant immunoglobulin present on all mucosal surfaces, where it plays crucial roles in mucosal protection[3]. IgA is produced and released as a J chain-linked dimer by resident IgA+ plasma cells in mucosal tissues, including the extensive lamina propria of the intestine[22]. For decades, it has already been known that IgA plays a protective role in mucosal immunity. IgA exerts its protective effects *via* 3 primary mechanisms. First, IgA serves as an immunologic barrier which inhibits binding of organisms to mucosal surfaces. Next, the normal movement of IgA from the basilar to apical region of epithelial cells suggests that it may be effective in neutralizing intracellular pathogens. Finally, pathogens bound to IgA are taken up by macrophages *via* phagocytosis[23]. An additional property of IgA is its inability to trigger the release

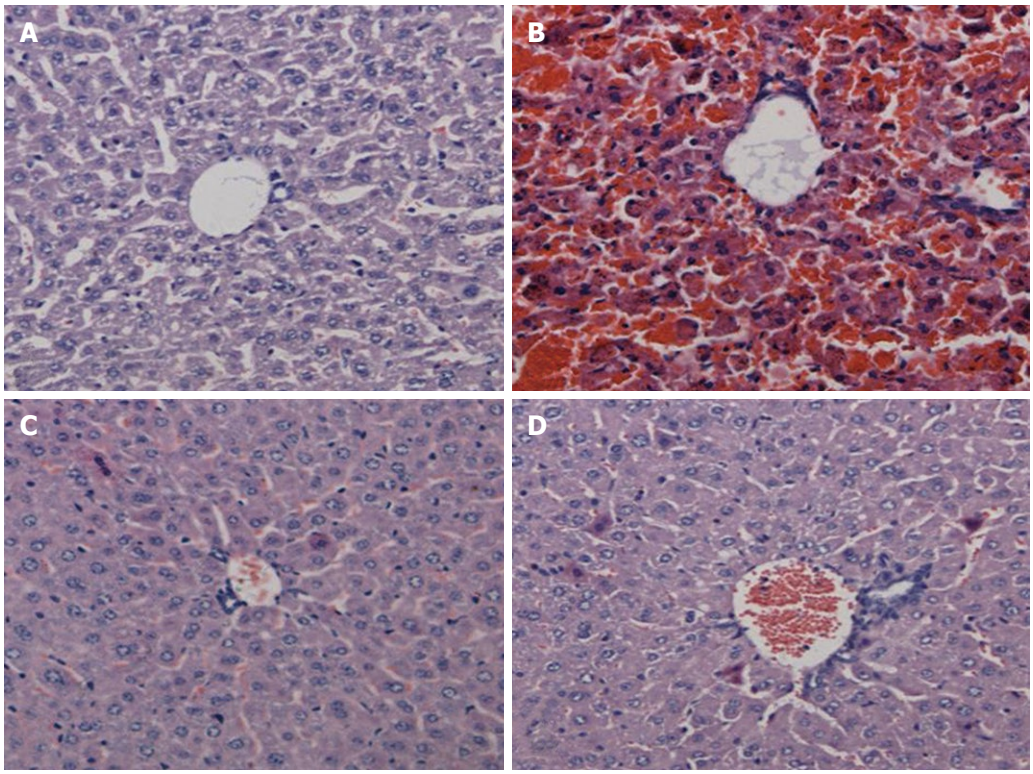

Figure 2 Hematoxylin and eosin staining of liver tissue (100 ×). A: Normal group; B: Acute liver necrosis group; C: Lipopolysaccharide group; D: Galactosamine group.

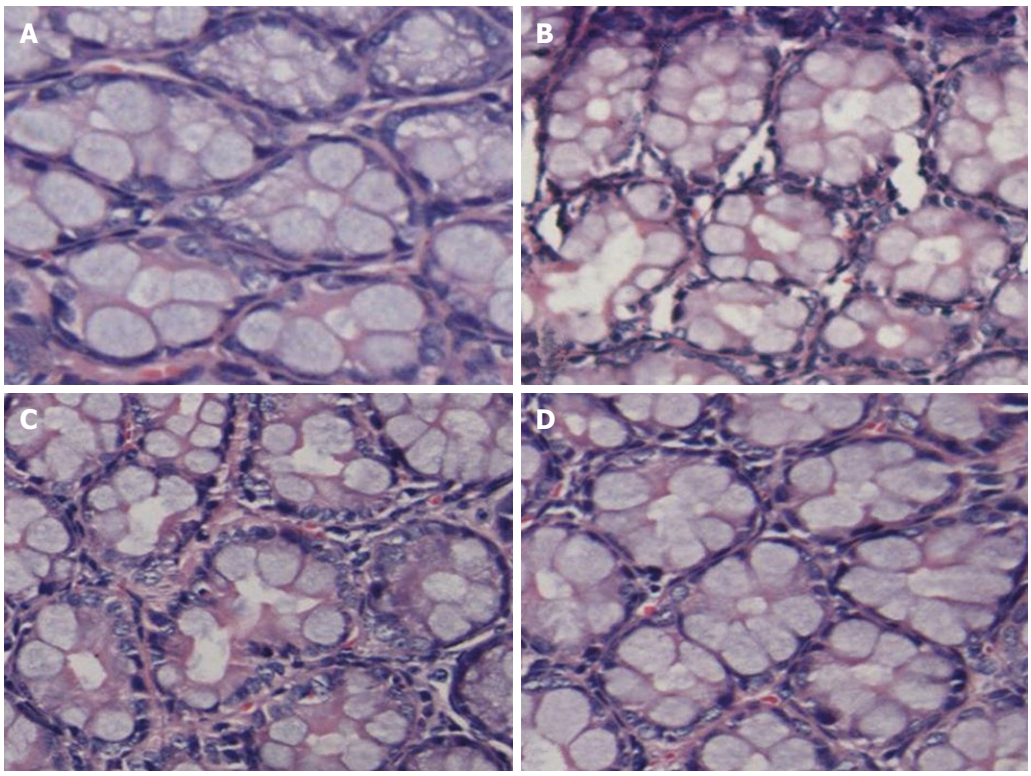

Figure 3 Morphology of intestinal samples stained with hematoxylin and eosin (200 ×). A: Normal group; B: Acute liver necrosis group; C: Lipopolysaccharide group; D: Galactosamine group.

of inflammatory mediators through receptors specific to its Fc domain[24-26].

IgA-deficient individuals have a tendency to develop infections and disorders of the gastrointestinal tract[27-29]. Zinneman *et al*[30] reported that the protective barrier of the gastrointestinal system was impaired in IgA deficiency

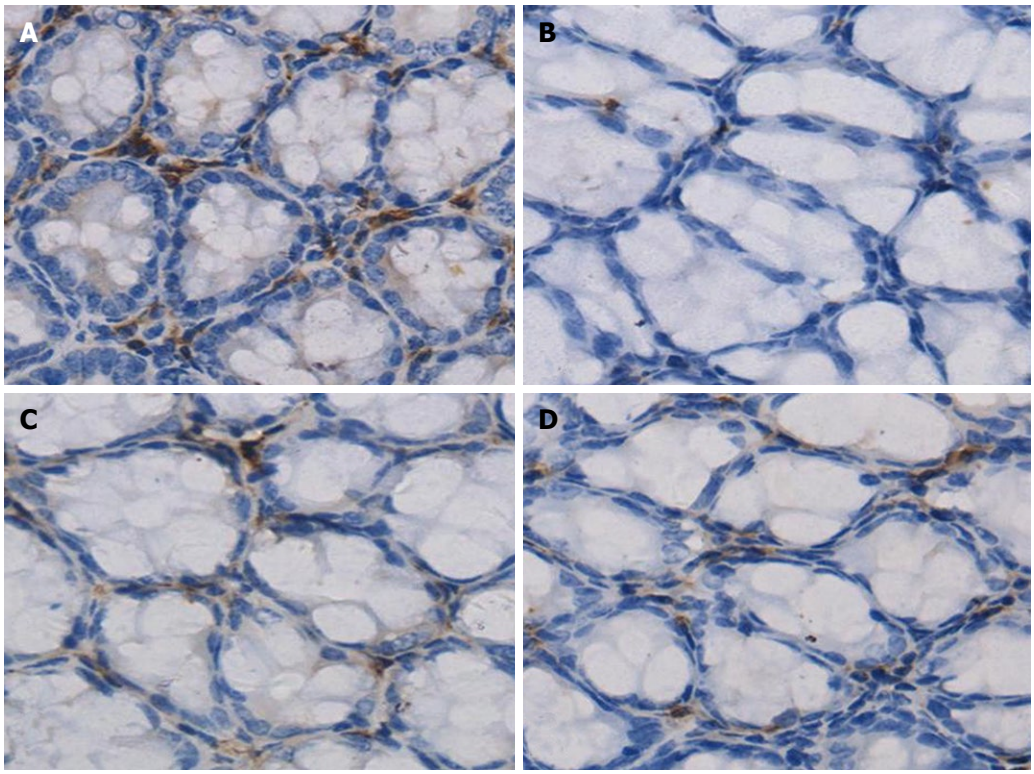

Figure 4 Immunoglobulin A+ cells determined by immunohistochemistry (400 ×). Immunohistochemistry demonstrated that Immunoglobulin A in the plasma cells within the lamina propria were stained brown. A: Normal group; B: Acute liver necrosis group; C: Lipopolysaccharide group; D: Galactosamine group.

and that protozoa such as *Giardia lamblia* can adhere to the epithelium, proliferate, and cause infection.

In the experiment presented herein, the number of IgA+ plasma cells and the IgA levels in intestinal mucosa in LPS and GalN groups showed a slight decrease but no significant difference was noted compared to the normal group. The number of IgA+ plasma cells and the IgA levels in the intestinal mucosa in the acute liver necrosis group were the lowest among all 4 study groups. The IgA+ plasma cells and the IgA levels were significantly different between the acute liver necrosis group and normal controls (Figures 5A and B). The decrease in the number of IgA+ cells and the IgA levels in the GalN/LPS group were significantly greater than the sum of the decrease in the LPS and GalN groups. It was thought that the decreased IgA+ plasma cells and decreased IgA levels in the intestinal mucosa were not the result of GalN or LPS injection, but rather of acute liver necrosis. These findings suggest that intestinal immunological barrier injury, which is a component of intestinal barrier injury, does occur in acute liver necrosis.

The mechanism of reduction in IgA+ plasma cells in acute liver necrosis is complicated and likely multifactorial. First, an increased rate of apoptosis in the subpopulation in Peyer's patches secondary to acute liver necrosis could negatively impact IgA+ plasma cell numbers[9,31]. Second, multiple organ damage, particularly the bone marrow, spleen, and mesenteric lymph nodes, in concert with mucosal edema and injury caused by acute liver necrosis, could affect the production and proliferation of IgA+ plasma cells. Third, the structural damage to the intestinal mucosa could interfere with recirculation of plasma cell precursors[32]. An accurate mechanism of IgA+ plasma cell reduction in acute liver necrosis clearly requires further study.

In this study, it was also found that the decreased IgA expression in acute liver necrosis coincided with a decline in IgA+ plasma cells. One explanation for this could be that the decrease in the number and function of IgA+ plasma cells leading to a simultaneous reduction in IgA secretion. At present, the specific pathogenesis and progression of acute liver necrosis remains unclear.

Inflammatory mediators are thought to be involved in the development and progression of acute liver necrosis. Previous studies reported that serum levels of a number of inflammatory factors, such as IFN-γ, are elevated in patients with severe liver injury[14,33]. In addition, IFN-γ is known to downregulate IgA expression[34].

In this study, IFN-γ levels in the small intestinal mucosa were slightly increased in LPS, GalN and acute liver necrosis groups, but no significant difference in IFN-γ expression was identified between the acute liver necrosis and normal control group. IFN-γ expression does not seem to explain the decrease in IgA secretion from the intestinal mucosa. Other factors involved in the reduction in IgA expression in acute liver necrosis warrant further attention.

In conclusion, this study found that mice with acute liver necrosis had a reduced number of intestinal IgA+ plasma cells and IgA expression levels indicating that mucosal immune barrier dysfunction does exist in acute

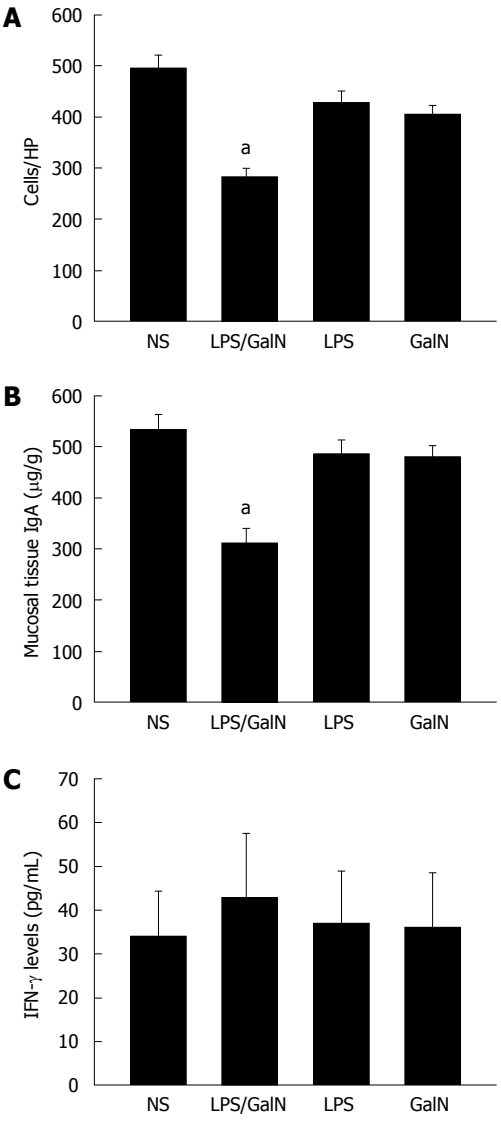

Figure 5 Immunoglobulin A+ plasma cells (A), the Immunoglobulin A expression levels (B) and interferon-γ expression (C) in intestinal mucosal tissue. $^{a}P < 0.05$ *vs* normal saline (NS). HP: High power field; GalN: Galactosamine; LPS: Lipopolysaccharide.

liver necrosis. IFN-γ expression does not seem to explain the decrease in IgA secretion from the intestinal mucosa. Further research regarding the mechanism(s) of intestinal immune barrier injury and ways to prevent this type of injury in acute liver necrosis is warranted.

ACKNOWLEDGMENTS

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COMMENTS

Background

Enterogenic infection is an important cause of death in patients with acute liver necrosis. Intestinal immunological barrier injury plays a vital role in the pathophysiology of enterogenic infection. Immunoglobulin A (IgA) is an important component of the intestinal immunological barrier and is the most abundant immunoglobulin present on mucosal surfaces, where it plays crucial roles in mucosal protection.

Research frontiers

IgA is considered a first line antigen-specific immune defense against pathogenic microorganisms and plays an important role in intestinal mucosal immunity. This study found significant changes in the number of IgA+ plasma cells and IgA expression levels during acute liver necrosis.

Innovations and breakthroughs

Previous studies have mainly focused on mechanical barrier interruption in acute liver necrosis models. So far, no studies have shown the indispensable nature of the intestinal immunological barrier in acute liver necrosis. In this study, the number of IgA+ plasma cells and IgA expression in mice with acute liver necrosis were determined to investigate whether dysfunction of the immunological barrier occurred during acute liver necrosis.

Applications

IgA is an important component of mucosal immune system and is significantly reduced during acute liver necrosis. Thus, a protective or an immunoregulative treatment of intestinal immune function could be beneficial in patients with acute liver necrosis.

Peer review

The manuscript by Fu *et al* describes studies examining in mice the change of intestinal IgA+ plasma cells and the expression of intestinal IgA in acute liver necrosis. Although the underlying mechanisms that contribute to the decreased expression of intestinal IgA was not thoroughly investigated, the study addresses an issue of topical interest, providing important data.

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BRIEF ARTICLE

Early ileocolonoscopy with biopsy for the evaluation of persistent post-transplantation diarrhea

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Abstract

AIM: To investigate the significance of ileocolonoscopy with histology in the evaluation of post-transplantation persistent diarrhea (PD).

METHODS: We retrospectively reviewed all records of renal transplant patients with PD, over a 3-year period. All patients were referred for ileocolonoscopy with biopsy, following a negative initial diagnostic work up. Clinical and epidemiological data were compared between cases with infectious or drug-induced diarrhea.

RESULTS: We identified 30 episodes of PD in 23 renal transplant patients (1-3 cases per patient). There were 16 male patients and the mean age at the time of PD was 51.4 years. The average time from transplantation to a PD episode was 62.3 ± 53.2 mo (range 1-199 mo). Ileocolonoscopy detected mucosal abnormalities in 19 cases, whereas the intestinal mucosa appeared normal in 11 cases. Histological examination achieved a specific diagnosis in 19/30 cases (63.3%). In nine out of 11 cases (82%) with normal endoscopic appearance of the mucosa, histological examination of blinded biopsies provided a specific diagnosis. The etiology of PD was infectious in 11 cases (36.6%), drug-related in 10 (33.3%), of other causes in three (10%), and of unknown origin in six cases (20%). Infectious diarrhea occurred in significantly longer intervals from transplantation compared to drug-related PD (85.5 ± 47.6 mo *vs* 40.5 ± 44.8 mo, $P < 0.05$). Accordingly, PD due to drug-toxicity was rarely seen after the first year post-transplantation. Clinical improvement followed therapeutic intervention in 90% of cases. Modification of immunosuppressive regimen was avoided in 57% of patients.

CONCLUSION: Early ileocolonoscopy with biopsies from both affected and normal mucosa is an important adjunctive tool for the etiological diagnosis of PD in renal transplant patients.

Key words: Endoscopy; Post-transplantation diarrhea; Histology; Enteric infections; Mycophenolate mofetil-colitis

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INTRODUCTION

Diarrhea occurs frequently following renal transplantation, with reported incidences as high as 64% in large clinical trials[1-3]. Although several cases are benign and easily manageable, post-transplantation diarrhea can persist for a long period and compromise the health status of the patients. In particular, it leads to water and electrolyte disturbances, interferes with the absorption of immunosuppressive drugs, often requires hospital admission, and thus negatively affects the quality of life of the patients[4]. An association between post-transplantation diarrhea and decreased graft and patient survival has also been reported[5].

The diagnostic algorithm of post-transplantation diarrhea should take into consideration the specific characteristics of this population, particularly the presence of significant immunosuppression[6,7]. Infectious agents are often implicated; however, manifestation of enteric infections can vary considerably in this population[8,9]. Atypical presentations and severe forms of common infections frequently occur, whereas opportunistic infections with unusual microorganisms are also encountered. On the other hand, immunosuppressive regimens can cause intense and persistent diarrhea (PD)[10]. The most prominent example is toxicity of mycophenolate mofetil (MMF), which can cause enterocolitis in a substantial proportion of patients[11-14], requiring modification of the immunosuppressive regimen. However, reducing the dose of immunosuppression might lead to graft loss[15].

In the present study we have analyzed all cases of PD in renal transplant patients in our Hospital between July 2006 and June 2009. Our aim was to investigate the utility of early ileocolonoscopy, with biopsies taken both from identified lesions and blindly from normal looking mucosa, in establishing a definitive diagnosis for the diarrheal episode.

MATERIALS AND METHODS

Patient population and definitions of PD

We retrospectively reviewed the records of all renal transplant patients who presented with PD and had ileocolonoscopy as part of their diagnostic work-up in our hospital between July 2006 and June 2009.

All patients were followed at the Renal Transplantation Unit of our Hospital. Demographic, epidemiological, and clinical characteristics of the patients at the time of each diarrheal episode were retrieved from the medical files. We defined PD as an episode of diarrhea with the following characteristics: (1) change in the bowel habits with more than three movements per day and decreased stool consistency lasting longer than 2 wk; (2) an etiological diagnosis was not established after initial testing, including detailed history and clinical examination, extensive hematological, and biochemical tests, as well as stool cultures for enteric pathogens, examination for ova and parasites, and examination for *Clostridium difficile* toxins-A and B; (3) failure of diarrhea to resolve following simple dietetic modifications and non-immunosuppressive medication adjustment; and (4) further testing including ileocolonoscopy was considered necessary by the attending nephrologist, because diarrhea interfered with health status and quality of life of the patient. All patients with PD were tested with polymerase chain reaction (PCR) for cytomegalovirus (CMV) in blood; however, colonoscopy was always performed to detect endoscopic and/or histologically evident CMV-colitis.

Over the 3-year study period there was an agreed standard practice between the Renal Transplantation Unit and G.I. Endoscopy Unit of the 1st Department of Internal Medicine, to which renal transplant patients with PD are referred for ileocolonoscopy. Polyethylene glycol was used for bowel preparation. Sodium phosphate-based regimens were avoided due to their reported nephrotoxicity. Colonoscopy was performed with sedation (midazolam) and analgesia (pethidine), as required. During endoscopy, multiple biopsies were taken from all areas with mucosal abnormalities as well as blind biopsies from normal looking mucosa of the terminal ileum and throughout the colon (4-6 biopsies from right and left colon, respectively). Upper gastrointestinal (GI) tract endoscopy was performed selectively according to the clinical judgment of the treating physicians.

We defined the following categories of PD in relation with the underlying cause: (1) infectious, when a microorganism with an established role as a diarrhea-causing agent was detected by microbiological, histological, or molecular methods; (2) drug-induced, when infectious agents were excluded and histological findings consistent with pharmaceutical injury (most often MMF-related) were detected in the biopsy specimens. Histological findings highly suggestive of MMF-colitis, included: (a) mucosal abnormalities characterized by atrophy, crypt architectural distortion, flattened crypt epithelium, increased cell apoptosis and regenerative epithelial changes; and (b) edema, moderate inflammatory infiltrations with increased number of eosinophils, crypt abscesses and cryptitis, and, in the more severe cases, focal erosions or ulceration[13]. In addition, a clear beneficial effect of modification of the immunosuppressive regimen (MMF-dose reduction or switching to Myfortic or azathioprine) on the severity of PD was required to confirm a drug (MMF)-associated etiology of diarrhea; (3) Other, when a definitive cause (not associated with immunosuppressive medications or infectious agents) was established by clinical, laboratory, and histological findings; and (4) unknown, when no causative factor was identified. This group included cases with nonspecific changes either in endoscopy and/or at histology.

Statistical analysis

The SPSS software was used for the analysis. Continuous variables were analyzed by the independent *t*-test or

Table 1 Clinical and demographic characteristics of the study population[1]

	Total	Drug	Infection	Non-drug, non-infectious[2]	*P*[3]
No. of cases of persistent diarrhea	30	10	11	9	
Gender, *n* (%)					
Female	8 (26.7)	3 (30)	2 (18.2)	3 (33.3)	
Male	22 (73.3)	7 (70)	9 (81.8)	6 (66.7)	
Donor type					
Cadaveric	43.3	60	27.3	44.4	
Living	56.7	40	72.7	55.6	NS
Age at diarrheal episode (yr), mean ± SD (range)	51.4 ± 15.5 (24-76)	46.9 ± 17.1 (27-76)	52.6 ± 10 (40-70)	54.8 ± 19.4 (24-75)	NS
History of previous diarrheal episode, *n* (%)	21 (70)	4 (40)	9 (81.8)	8 (89)	0.081
Time since transplantation (mo), mean ± SD (range)	62.3 ± 53.2 (1-199)	40.5 ± 44.8 (1-142)	85.5 ± 47.6 (2-179)	58.1 ± 61.8 (6-199)	0.038
Immunosuppressive regimen[4], *n* (%)					
Mycophenolate mofetil + tacrolimus	18 (60)	6 (60)	6 (54.5)		
Mycophenolate mofetil + cyclosporine	2 (6.6)		1 (9.1)		
Mycophenolate mofetil + sirolimus	2 (6.6)	1 (10)	1 (9.1)		
Mycophenolate mofetil + everolimus	1 (3.3)	1 (10)			
Everolimus + tacrolimus	2 (6.6)		2 (18.2)		
Tacrolimus	2 (6.6)				
Mycophenolate sodium + tacrolimus	3 (10)	2 (20)	1 (9.1)		
Hospital stay (d), mean ± SD (range)	18.1 ± 30.6 (0-169)	8.5 ± 8.5 (0-22)	17.4 ± 11.6 (0-37)	29.4 ± 53.8 (0-169)	0.076
Outcome, *n* (%)					
Cessation of diarrhea	22 (73.3)	9 (90)	9 (82)	4 (44.4)	NS
Improvement	5 (16.7)			5 (55.6)	
Death/graft loss	3 (10)	1 (10)	2 (18)		

[1]Data are presented per episode of persistent diarrhea; [2]Other and unknown groups combined; [3]Comparison between infectious and drug-induced cases of persistent diarrhea; [4]All patients were taking methylprednisolone at the time of persistent diarrhea. NS: Not significant.

Mann-Whitney test (if they did not meet the criteria for parametric comparison). Categorical variables were studied by corrected χ^2 test. For all comparisons a probability level (*P*) of 0.05 was considered significant.

RESULTS

Demographic data

Over the study period, 30 ileocolonoscopies were performed for 30 separate episodes of PD in 23 renal transplant patients (Table 1). One patient had three episodes, five had two, and seventeen patients had one episode of PD. In all but one patient, the cause of PD differed between separate episodes. There was a clear predominance of males (2.3:1 male/female ratio), independently of the etiology of diarrhea (Table 1). The cause of renal failure and transplantation was polycystic kidney disease in four patients, kidney stone disease in three, whereas Henoch-Schönlein purpura, IgA nephropathy, recurrent kidney infections, renal hypoplasia, medullary cystic disease, and polyarteritis nodosa accounted for one case each. The etiology was unknown in 10 patients.

The immunosuppressive regimens that were administered at the time of each case of PD are shown in Table 1. All patients with more than one episode of PD were receiving the same immunosuppressive medications in all episodes, with the exception of one patient who was switched from MMF/tacrolimus (1st episode) to everolimus/tacrolimus (2nd and 3rd episodes) and a second patient in whom MMF/tacrolimus was changed to mycophenolate sodium/tacrolimus.

Table 2 Endoscopic[1] and histological[2] findings in renal transplant patients with persistent diarrhea

Cases	Endoscopy + histology +	Endoscopy + histology -	Endoscopy - histology +	Endoscopy - histology -
All	16	3	9	2
Drug-induced	4		6	
Infectious	7	1[3]	3	
Other	3			
No diagnosis[4]	2	2	0	2

[1]Ileocolonoscopy; [2]Including biopsies from normal-looking mucosa; [3]In this case biopsy was not taken because of typical pseudomembranous colitis in endoscopy; [4]Including cases with non-specific colitis in endoscopy or histology.

Endoscopic and histological studies

Twenty endoscopies were performed in inpatients and ten in outpatients. The cecum was reached in 26/30 colonoscopies (86.7%), with terminal ileum intubation in the vast majority of cases (22/26 with cecum intubation, 85%). We did not observe any serious complications related either to the preparation for colonoscopy, the use of sedatives/analgesics, or the procedure itself.

The diagnostic yield of ileocolonoscopy and histological examination of endoscopically obtained intestinal specimens are shown in Table 2. Biopsies were taken in all but one patient, in whom diagnosis of pseudomembranous colitis was established by typical history of prior antibiotic administration and endoscopic findings. Ileocolonoscopy revealed mucosal abnormalities in 2/3 of the patients. The most frequently encountered findings were

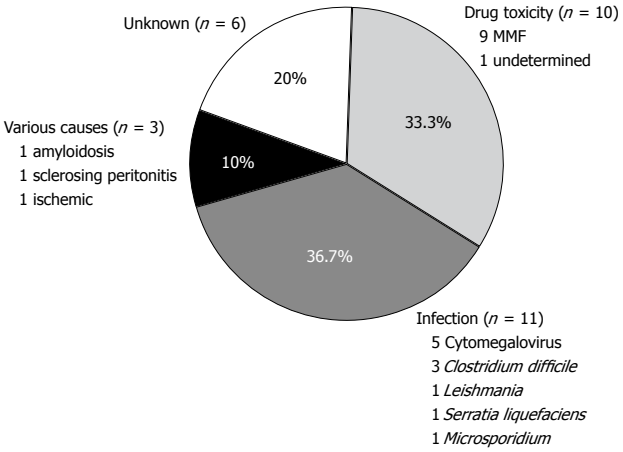

Figure 1 Causes of persistent diarrhea in renal transplant patients. MMF: Mycophenolate mofetil.

edema (loss of submucosal vascular pattern) and erythema of the mucosa, which were observed in 11 cases. More severe lesions included colonic ulceration (three cases), stenosis (two cases), submucosal hemorrhage (one case), and formation of pseudomembranes (two cases). We did not observe any endoscopic findings that were exclusively associated with infectious or drug-induced diarrhea (with the exception of pseudomembranous colitis).

Histological examination of biopsies obtained during endoscopy provided a definitive diagnosis in 19/30 cases (63.3%). More importantly, histology allowed for a specific diagnosis in nine out of 11 cases with normal endoscopic examinations (Table 2). Overall, an infectious cause was identified in 11 cases (Figure 1). The most prevalent infection was due to CMV, accounting for 16.6% of all cases. Interestingly, in 3/11 infectious cases (27%) there were no mucosal abnormalities seen on endoscopy. In three cases, diagnosis was established histologically in biopsy specimens taken from areas of normal looking mucosa (Table 2). These included two cases of CMV infection and one case infected with microsporidium. A case of leishmaniasis was diagnosed histologically by the recognition of the dot-like organisms within mucosal macrophages (Figure 2C). These were also revealed by Giemsa stain while PAS stain was negative.

In our study, we identified 10 episodes of PD (33.3%) that were related to toxicity of immunosuppressive drugs (Figure 1). All patients with drug-related diarrhea were receiving mycophenolate (eight MMF and two mycophenolate sodium) in combination with tacrolimus (eight cases), everolimus (one case), or sirolimus (one case). In the majority of drug-induced PD (6/10, 60%) the colonic mucosa looked normal on colonoscopy. Nevertheless, histological evaluation of blindly collected biopsies revealed mucosal changes consistent with MMF-colitis in all cases; thus establishing the diagnosis of drug-induced injury. These findings included mucosal abnormalities such as edema, atrophy, crypt architectural distortion, regenerative epithelial changes, and increased cell apoptosis with intraluminal apoptotic bodies (Figure 2A and B).

In our study there were three cases where a definitive diagnosis unrelated to infection or drug-toxicity was established. In the first patient, intestinal amyloidosis was diagnosed by histological examination and appropriate staining of a biopsy specimen obtained from the rectum. The second case involved a patient with sclerosing peritonitis. The pathophysiology of diarrhea was associated with external compression of the intestine by the sclerotic tissue and the accompanying motility and structural abnormalities, as diarrhea was completely abrogated following effective surgical decompression. Finally, in the third case, diarrhea was considered of ischemic origin as no other etiology was found and histology was compatible with ischemic intestinal injury. Taken together, these results show that endoscopy with histological examination of both affected and normal mucosa achieves a definitive diagnosis in the vast majority of PD in renal transplant patients.

Comparison between infectious and drug-induced PD

As our initial analysis showed that the majority of cases with PD were of infectious or pharmaceutical etiology, we then compared these two distinct groups for several characteristics. We observed no association between the type of diarrhea and the gender or age of the patient, or the type of donor (cadaveric *vs* living) (Table 1). In contrast, the time from transplantation to the PD episode differed significantly according to the etiological factor. In particular, this interval was considerably shorter in drug-related (40.5 ± 44.8 mo), as compared to infectious diarrhea (85.5 ± 47.6 mo, $P < 0.05$). There was a statistically significant difference between infectious and drug-induced PD ($P < 0.05$) in regards to their temporal distribution (Figure 3). In particular, while all but one case of infectious PD (91%) occurred later than 4 years post-transplantation, drug toxicity was usually seen at earlier time points. Accordingly, infection accounted for 14% of early episodes, whereas pharmaceutical toxicity accounted for 57%. In contrast, late episodes were caused primarily by infections (56%) and rarely by drugs (16.6%). In all, these results indicate that the time post-transplantation should be taken into consideration when searching for the etiology of PD in renal transplant patients, as different causes underlie early *vs* late episodes.

Outcome of PD

All but one case of infectious diarrhea required admission to the hospital, (91% admission rate) (Table 1). In contrast, fewer patients with drug-induced PD were admitted (60% admission rate). There was a trend towards longer hospital stay for patients with infectious diarrhea (mean hospital stay: 17.4 ± 11.6 d *vs* 8.5 ± 8.5 d for the drug-induced group, $P = 0.076$) (Table 1).

The overall outcome of PD was good, with cessation or improvement of diarrhea in 90% of cases (Table 1). There were two deaths in the infectious group, both unrelated to diarrhea. One patient with pseudomembranous colitis had a complicated clinical course due to disseminat-

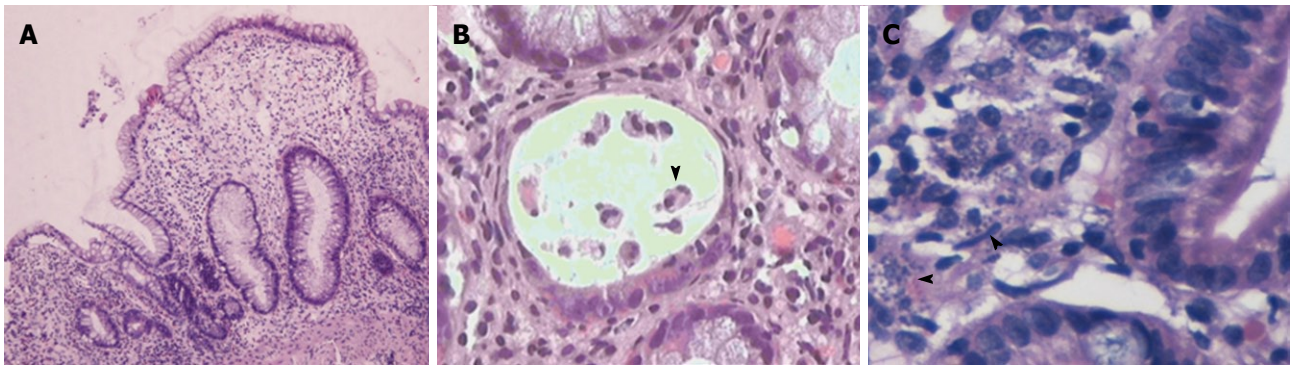

Figure 2 Histological photomicrographs from drug-induced and infectious cases of persistent diarrhea. A: Mycophenolate mofetil (MMF)-colitis, (HE stain, 200 × original magnification); B: MMF-colitis, with apoptotic bodies within the bowel lumen (arrowhead, HE stain, 400 × original magnification); C: Intestinal leishmaniasis with characteristic dot-like microorganisms within macrophages in the lamina propria (arrowheads, HE stain, 400 × original magnification).

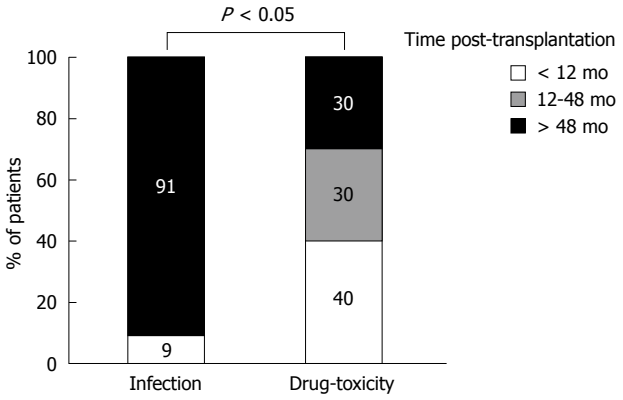

Figure 3 Distribution of infectious and drug-induced cases of persistent diarrhea in renal transplant patients according to the time post-transplantation.

ed fungal infection and was transferred to ICU where he eventually died. The other patient suffered from visceral leishmaniasis that had a fatal outcome.

Modification of immunosuppressive regimen was introduced in 12 cases. In five there was a switch from MMF to enteric-coated mycophenolate sodium, whereas in two the dose of mycophenolate was decreased with favorable outcomes in all cases. In four occasions mycophenolate had to be replaced by azathioprine. Finally, in one patient with drug-induced PD, diarrhea proved to be self-limited and required no change of immunosuppression. In our study, there was one case of graft loss in a patient with severe immunosuppression-related complications who had to stop all drugs with eventual loss of the graft.

DISCUSSION

In the present study we demonstrated that early ileocolonoscopy combined with histology of bowel mucosa, even without macroscopic abnormalities, is a critical component of the diagnostic evaluation of PD in renal transplant patients. We have shown that this approach provides a definitive diagnosis in the majority of cases, allowing prompt and specific treatment of the underlying cause, avoiding unnecessary modifications of the immunosuppressive regimen, and leading to favorable patient and graft outcome. Our data also indicate that PD is more likely due to drug-associated toxicity during the first post-transplantation year, while infectious diarrhea may occur throughout the post-transplantation period and is usually the cause of diarrhea after 4-year post-transplantation.

The majority of published studies on post-transplantation diarrhea did not take into account the severity or the duration of the episode[1-3,5]. In our study we focused on diarrhea that was judged as persistent, both in terms of long duration as well as of interference with the wellbeing of patients. We believe that these are the most clinically relevant cases and require extensive evaluation for the underlying causative agent. Our findings clearly show that there should be a low threshold for early ileocolonoscopy with histological examination in these patients. Such an approach is supported by the high percentage (80%) of definitive diagnoses that was accomplished in our study.

In a recent publication, a diagnostic algorithm for post-transplantation diarrhea was proposed, which introduced colonoscopy late in the course of evaluation and, more significantly, after modifications in immunosuppressive drugs were applied[16]. In fact, reduction of MMF is among the first measures taken in patients with post-transplant diarrhea[17]. This leads to cessation of diarrhea in a considerable proportion of cases, therefore avoiding the need for invasive tests such as colonoscopy. On the other hand, reducing the dose of immunosuppression often results in graft dysfunction[15,18,19]. In fact, in our study, the single episode of graft loss was associated with immunosuppression cessation due to severe toxicity, including drug-induced-diarrhea. Our results support the use of early endoscopy with histology in prolonged or refractory cases of diarrhea, as we were able to document non-drug-related causes in 46% of cases, thus avoiding unnecessary modifications of immunosuppressive regimens.

Early colonoscopy was suggested in a recent study on post-transplantation diarrhea, when there is strong clinical suspicion for CMV-colitis[20], including cases with positive PCR for CMV in the blood. Our findings support the use of colonoscopy with histology in this population, as it helps in establishing the localization of CMV in the intes-

tine and provides causality for chronic diarrhea. In fact, in our series, one of five cases with CMV-colitis had negative CMV-PCR in the blood, and a second one had very low number of CMV-DNA copies. Moreover, in some cases with positive CMV-PCR in the blood, colonoscopy and histology indicated absence of CMV-colitis, despite the presence of diarrhea, which was attributed to other causes.

To our knowledge there is only one published study that reported on the role of colonoscopy in renal transplant patients with diarrhea. Contrary to our study, Korkmaz *et al*[21] showed a 55% failure to establish a diagnosis with colonoscopy and/or histology. The higher rates observed in our study might be attributed to several factors. First, the severity of diarrhea in the Korkmaz study is not reported; it might, therefore, be the case that some colonoscopies were performed in milder cases with no obvious causative agent. Second, the accumulated experience on the histological lesions of MMF-colitis allowed us to use better-defined criteria for drug-induced toxicity; it is possible that such cases are included in the large number of non-specific colitis cases in the study by Korkmaz *et al*[21]. Finally, we took blinded biopsies in every patient, which was not the case in the aforementioned study. In another recent study only apparent lesions were biopsied during colonoscopy[22]. Our data clearly showed that histology of normal-appearing mucosa revealed pathognomonic findings in a considerable percentage of renal transplant patients with PD. In our study, this approach yielded a diagnosis in 27% of infection-related and in 60% of drug-induced cases of diarrhea.

Infectious agents and drugs accounted for the majority of PD cases in our cohort. This is in line with previous studies[23,24]. We detected a significant difference between the two groups (infectious *vs* drug-induced) regarding the time they occurred post-transplantation. In particular, the majority of drug-induced cases took place in the first years following transplantation. This distinction has also been observed in other studies[16]. This may be explained by the fact that intolerance to immunosuppressive regimen is expected to occur within relatively short time after their initiation[25]. In contrast, in our study, intestinal infection was diagnosed later than 4 years post-transplantation, almost exclusively. A temporal distribution of various infections post-transplantation has been reported[26]. These data, as well as our present findings, indicate that the search for PD etiology should be tailored to the individual patient, taking into consideration the time post-transplantation. In the case of an episode that takes place long after transplantation, intensive search for infectious agents is primarily required.

We were not able to establish a diagnosis in 6/30 cases (20%), including four that were classified as non-specific colitis. Follow-up revealed that diarrhea ceased or was greatly improved, indicating that self-limited infections and/or unspecified pharmacotoxicity underlay these cases. In fact, in one case *Candida albicans* was isolated from the stools, whereas in two others CMV-viremia was detected. However, since a direct proof of causality was not established, we classified these cases as non-specific colitis and not infectious. Only in two cases of unknown etiology was modification of immunosuppressive regimen considered necessary.

In conclusion, our results indicate that ileocolonoscopy has an important impact in the management of renal transplant patients with PD and should be an adjunctive tool for the causative diagnosis of PD. Endoscopy should be considered only after initial measures have failed to induce clinical improvement. These measures may include adjustment of the immunosuppressive regimen, particularly when diarrhea manifests during the initial post-transplantation months as the prevalence of drug-related causes is increased during that period. In any case, biopsies should always be taken from the lower GI tract as histology achieves a definitive diagnosis in the majority of cases, even when the intestinal mucosa appears macroscopically normal. This approach may offer the opportunity for specific treatment and lead to improved outcomes following renal transplantation.

COMMENTS

Background

Diarrhea is among the most common complications in patients who receive renal transplants and has been associated with poor outcomes in terms of quality of life as well as graft and patient survival. Infectious agents often cause diarrhea due to the universal administration of immunosuppressive regimens in this population. Immunosuppressants can themselves cause significant gastrointestinal toxicity, the most prominent example being mycophenolate mofetil-enterocolitis.

Research frontiers

Previous studies have reported diagnostic algorithms for the evaluation of post-transplantation diarrhea. Endoscopic and histological studies of the lower gastrointestinal tract have been incorporated only at the late steps of diagnostic protocols, usually when extensive clinical and laboratory work-up has been negative and modifications in the immunosuppressive scheme have been ineffective to induce diarrhea cessation.

Innovations and breakthroughs

In the present study, the authors investigated the usefulness of a standard approach for the evaluation of persistent diarrhea (PD) in renal transplant patients, which utilized an early ileocolonoscopy, i.e. as soon as limited laboratory testing came back negative. Moreover, biopsies were routinely taken both from all identified lesions but also blindly from normal looking mucosa. The present study demonstrated a high efficacy of this diagnostic scheme in establishing a definitive diagnosis for the diarrheal episode.

Applications

The application of early ileocolonoscopy with standard tissue sampling may facilitate etiologic diagnosis and targeted treatment of PD in renal transplant patients; thus avoiding unnecessary changes in the immunosuppressive regimen. This approach may be of particular importance in the late post-transplantation period, when non-drug related causes of diarrhea are increased.

Terminology

PD: an episode of diarrhea lasting longer than 2 wk and interfering with the health status and quality of life of the renal transplant patient, for which an etiological diagnosis is not established after initial clinical examination, baseline hematological and biochemical tests, as well as stool tests for infectious causes and which did not respond to simple dietetic modifications and non-immunosuppressive medication adjustments.

Peer review

The authors investigated the role of colonoscopy on persistent post-transplantation diarrhea. The results indicated that colonoscopy is a valuable diagnostic tool for evaluating transplant recipients with PD. The paper is well written and data clearly presented. The work contributes to the understanding and guides management of this important complication despite the small number of patients and selection bias.

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BRIEF ARTICLE

Helicobacter infection concomitant with metabolic syndrome further increase risk of colorectal adenomas

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Abstract

AIM: To investigate the association of colorectal adenomas with both *Helicobacter pylori* (*H. pylori*) infection and metabolic syndrome.

METHODS: Using a cross-sectional hospital-based study, we analyzed physical examination data from 9311 healthy subjects with overnight physical examinations performed between January 2004 and December 2006. Examined data included gender, age, life style, anthropometric measurements, blood pressure, biochemical and hematological studies, *H. pylori* infection detected by esophagogastroduodenoscopy and biopsy urease tests, and colorectal adenomas detected with a complete total colonoscopy.

RESULTS: The prevalence values for *H. pylori* infection, metabolic syndrome, and colorectal adenoma were 39.2%, 18.7%, and 20.7%, respectively. Colorectal adenoma risk factors included male gender [odd ratio (OR): 2.005, 95% confidence interval (CI): 1.740-2.310, $P < 0.001$], advanced age (OR: 1.046, 95% CI: 1.040-1.052, $P < 0.001$), smoking (OR: 1.377, 95% CI: 1.146-1.654, $P = 0.001$), increased body fat (OR: 1.016, 95% CI: 1.007-1.026, $P = 0.001$), higher white blood cell count (OR: 1.038, 95% CI: 1.005-1.073, $P = 0.025$), *H. pylori* infection (OR: 1.366, 95% CI: 1.230-1.517, $P < 0.001$), and metabolic syndrome (OR: 1.408, 95% CI: 1.231-1.610, $P < 0.001$). In addition, concomitant *H. pylori* infection with metabolic syndrome further increased the probability of colorectal adenomas.

CONCLUSION: Our study revealed *H. pylori* infection with concomitant metabolic syndrome might further increase the risk of colorectal adenomas.

Key words: Biopsy urease test; Colorectal adenoma; Colorectal cancer; *Helicobacter pylori*; Metabolic syndrome

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INTRODUCTION

Colorectal cancer is an extremely common malignancy and one of the leading causes of cancer mortality worldwide. Colorectal adenoma is the premalignant lesion in colorectal cancer and develops into colorectal carcinoma

through the adenoma-to-carcinoma sequence[1]. The direct etiology of colorectal neoplasms is still unknown. However, previous epidemiological studies have identified family history, dietary factors, smoking, sedentary lifestyles, and alcohol consumption as potential contributors to colorectal neoplasm development[2]. Identification of the etiology of colorectal neoplasms might assist in the development of strategies targeted toward its prevention.

Helicobacter pylori (*H. pylori*) is a human pathogen that infects the gastric mucosa and causes inflammatory process that culminate in chronic gastritis, peptic ulceration, gastric lymphoma of mucosa-associated lymphoid tissue, and adenocarcinoma[3]. *H. pylori* is a gram-negative microaerophilic bacillus, and has been classified by the International Agency for Research on Cancer as a class I human carcinogen since 1994[4]. The role of *H. pylori* in colorectal carcinogenesis has been epidemiologically examined in recent decades; however, the association has remained inconclusive. Several studies have identified an association between *H. pylori* infection and colorectal neoplasms[5-9], while others have identified a negative association between the two[10-12]. Methodological issues might account for some of the inconsistent results, including the IgG serum antibody test and incomplete colonoscopic examinations for diagnosis.

Metabolic syndrome is a clinical cluster of metabolic abnormalities. It is also referred to as insulin resistance syndrome, and is diagnosed by criteria corresponding to the modified National Cholesterol Education Program (NCEP) criteria[13]. Diagnosis is fulfilled by the presence of any three of the following conditions: higher waist circumference ($\geq$ 90 cm in men and $\geq$ 80 cm in women), elevated triglycerides ($\geq$ 150 mg/dL), lower high density lipoprotein cholesterol ($<$ 40 mg/dL in men and $<$ 50 mg/dL in women), elevated blood pressure (systolic blood pressure $\geq$ 130 mmHg or diastolic blood pressure $\geq$ 85 mmHg), and elevated fasting glucose ($\geq$ 100 mg/dL). This syndrome might be a risk factor for type 2 diabetes and cardiovascular disease[14,15]. In recent years, metabolic syndrome has also been associated with an increased risk of colorectal adenoma. However, there is very limited medical literature examining the relationship between colorectal adenoma and metabolic syndrome[16-18]. Additional information on the correlation between metabolic syndrome and colorectal neoplasms could result in the recommendation for screening of colorectal neoplasms in the patient with metabolic syndrome.

Using a cross-sectional hospital-based study, we investigated the association of colorectal adenoma with both *H. pylori* infection and metabolic syndrome. Further, the probability of colorectal adenoma in patients with both *H. pylori* infection and metabolic syndrome was evaluated.

MATERIALS AND METHODS

A total of 11 787 asymptomatic subjects were admitted to the general physical examination department of the Buddhist Dalin Tzu-Chi General Hospital for general check-ups (two-day health examination) between January 2004 and December 2006. Excluding 2476 subjects aged below 40 years, a final total of 9311 study participants (3906 males and 5405 females) were enrolled in the study. The demographic data included age, gender, medical past history, and lifestyle. Clinical data included blood pressure, fasting plasma sugar, plasma lipids levels (total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, and triglycerides), and hematological variables. Anthropometric measurements including height (meters), weight (kilograms), and body fat (percent; Body Composition Analyzer TBF-410, Tanita, Japan) were also examined.

Metabolic syndrome was diagnosed with the modified NCEP criteria. *H. pylori* infection was detected by the biopsy urease test (CLO test, Pronto Dry, Gastrex, Poland) using standard video esophagogastroduodenoscopy (EGD) with gastrofibroscopes (GIFXP-240, GIFQ260, Olympus Optical, Tokyo Japan). A specimen for biopsy urease testing of each subject was taken from the gastric antrum using biopsy forceps and assessed within 60 min. The agar color of the biopsy urease testing turned from yellow to red when the biopsy specimen was infected with *H. pylori*, which contained intracytoplasmic urease. Colorectal adenomas were identified by complete total colonoscopy using standard video colonoscopes (CF 240I, Olympus Optical, Tokyo, Japan) by single- and double-handed methods under intravenous 1% Propofol (Fresenius Kabi, Austria). This study was performed under the approval of our hospital Institutional Review Board.

Statistical analysis

Data for continuous variables were expressed as mean ± SD. The *t* test was applied for continuous variables when the data fitted a Gaussian distribution. If the continuous data did not fit the Gaussian distribution, the Wilcoxon rank sum test was applied. Categorical variables were tested with the χ^2 test. Stepwise logistic regression analysis was conducted for significant variable selection. Basic model-fitting techniques for regression analysis were applied to assure the quality of analysis results, including variable selection, goodness-of-fit assessment, and regression diagnostics. Statistical significance was established for two-sided *P* values $<$ 0.05. All statistical analyses were performed with the SAS® software, version 9.1.3 (SAS Institute Inc., Cary, NC, USA) and R 2.6.2 (R Development Core Team, R Foundation for Statistical Computing, 2008, Vienna, Austria).

RESULTS

The median ages of the study participants were 54 years in males and 52 years in females. All subjects went through complete EGD examination, and 2.8% of participants had incomplete colonoscopy examination. The raw prevalence rates of *H. pylori* infection, metabolic syndrome, and colorectal adenoma were 39.2%, 18.7% and 20.7%,

Table 1 Baseline characteristics of the study subjects

Variable	Male	Female	*P*
n	3906	5405	
Age (yr)	54 (48, 61)	52 (47, 59)	< 0.001
Smoke, *n* (%)	884 (22.6)	49 (0.9)	< 0.001
Alcohol, *n* (%)	876 (22.4)	119 (2.2)	< 0.001
Body weight (kg)	67.6 (61.8, 74.2)	56.0 (51.3, 61.7)	< 0.001
Body fat (%)	22.7 (19.4, 26.1)	30.6 (26.8, 34.9)	< 0.001
Systolic BP (mmHg)	128 (116, 141)	122 (111, 138)	< 0.001
Diastolic BP (mmHg)	81 (73, 88)	74 (66, 82)	< 0.001
Hypertension, *n* (%)	1518 (38.9)	1575 (29.1)	< 0.001
Diabetes, *n* (%)	317 (8.1)	330 (6.1)	< 0.001
Glucose AC (mg/dL)	90 (84, 97)	88 (83, 95)	< 0.001
TCH (mg/dL)	191 (168, 215)	190 (169, 215)	0.448
WBC (× 10^3/μL)	6.31 (5.38, 7.42)	5.90 (5.02, 6.97)	< 0.001
Lymphocyte (%)	32.2 (27.0, 37.4)	33.9 (28.7, 39.2)	< 0.001
MS, *n* (%)	755 (19.3)	982 (18.2)	0.154
H. pylori, *n* (%)	1571 (40.2)	2083 (38.5)	0.096
Adenoma, *n* (%)	1053 (27.0)	870 (16.1)	< 0.001

n: Subject number; BP: Blood pressure; TCH: Total plasma cholesterol; WBC: White blood cell; *H. pylori*: *Helicobacter pylori*; MS: Metabolic syndrome.

Table 2 Multivariate logistic regression analysis of the risk factors for colorectal adenomas

Variable	β	SE	*P*	OR	95% CI
Intercept	-5.031	0.253	< 0.001	-	-
Gender (M *vs* F)	0.696	0.072	< 0.001	2.005	1.740-2.310
Age (per year)	0.045	0.003	< 0.001	1.046	1.040-1.052
Smoke (yes *vs* no)	0.320	0.094	0.001	1.377	1.146-1.654
Alcohol (yes *vs* no)	-0.010	0.093	0.915	0.990	0.826-1.187
Body fat (%)	0.016	0.005	0.001	1.016	1.007-1.026
WBC (per 10^3/μL)	0.038	0.017	0.025	1.038	1.005-1.073
H. pylori (yes *vs* no)	0.312	0.054	< 0.001	1.366	1.230-1.517
MS (yes *vs* no)	0.342	0.068	< 0.001	1.408	1.231-1.610

WBC: White blood cell; *H. pylori*: *Helicobacter pylori*; MS: Metabolic syndrome; OR: Odd ratio; CI: Confidence interval.

respectively. A total of 1923 adenomas, including 1691 tubular adenoma, 208 tubulovillous adenomas, and 24 serrated adenomas, were detected. Males were significantly older ($P < 0.001$), were more likely to smoke ($P < 0.001$), drink alcohol ($P < 0.001$), have heavier body weight ($P < 0.001$), lesser body fat ($P < 0.001$), and higher systolic and diastolic blood pressure values ($P < 0.001$). Males additionally had a higher proportion of hypertension ($P < 0.001$), diabetes ($P < 0.001$), higher fasting blood glucose levels ($P < 0.001$), higher white blood cell (WBC) counts ($P < 0.001$), lower lymphocyte percentages ($P < 0.001$), and a higher prevalence of colorectal adenoma ($P < 0.001$). There were no significant differences in total plasma cholesterol levels ($P = 0.448$), metabolic syndrome frequency ($P = 0.154$), and *H. pylori* infection frequency ($P = 0.096$) between males and females (Table 1).

Multivariate logistic regression analysis revealed that male gender (OR: 2.005; 95% confidence interval (CI), 1.740-2.310, $P < 0.001$), advanced age (OR: 1.046, 95% CI: 1.040-1.052, $P < 0.001$), smoking (OR: 1.377, 95% CI: 1.146-1.654, $P = 0.001$), increased body fat (OR: 1.016, 95% CI: 1.007-1.026, $P = 0.001$), higher white blood cell (WBC) count (OR: 1.038, 95% CI: 1.005-1.073, $P = 0.025$), *H. pylori* infection (OR: 1.366, 95% CI: 1.230-1.517, $P < 0.001$), and metabolic syndrome (OR: 1.408, 95% CI: 1.231-1.610, $P < 0.001$) were associated risk factors for colorectal adenoma. Alcohol consumption (OR: 0.990, 95% CI: 0.826-1.187, $P = 0.915$) was not a risk factor for colorectal adenoma (Table 2). Under analysis with a conditional effect plot, colorectal adenoma risk was positively associated with WBC count among paired groups of positive and negative *H. pylori*-infected patients and paired groups of positive and negative metabolic syndrome patients (Figure 1).

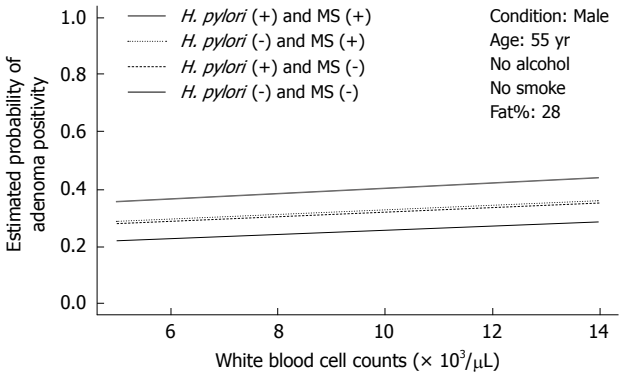

Figure 1 Conditional effect plot of *Helicobacter pylori* infection status and metabolic syndrome on the probability of adenoma positivity. The conditions were designed as non-smoking males at 55 years old and 28% body fat with the pair of both *Helicobacter pylori* (*H. pylori*) positivity and metabolic syndrome (MS) positivity *vs* another three pairs.

DISCUSSION

The results of our study supported the association of *H. pylori* infection with colorectal adenomas and were consistent with previous reports that metabolic syndrome might increase colorectal adenoma risk. It also showed that individuals with concomitant metabolic syndrome and *H. pylori* infection might have a further increased risk of colorectal adenomas.

The inconclusive results of previous studies concerning the relationship between *H. pylori* infection and colorectal neoplasm might have been due to sample bias, small sample size, inadequate consideration of potential confounding variables, and a varying frequency of cag A+ strains in the study populations[19,20]. In addition, incomplete colonoscopic studies and evaluation of *H. pylori* infection with the IgG serum test (which cannot represent real-time *H. pylori* infection) might also have contributed to the inconsistent results. The advantages of our study include large sample size, detection of *H. pylori* infection with the EGD and biopsy CLO test, and complete colonoscopy to the distal terminal ileum after good bowel preparation in 97.2% of the cases. Furthermore, patient lifestyle habits including smoking and alcohol consumption, gender, and age were also evaluated in this study. These factors might minimize potential variables during the data analysis.

However, there were also some limitations in our study. Patients in the study were selected from a population who sought routine physical examinations at our institute, and their psychosocial behaviors and lifestyle habits might differ from those in the general population, resulting in a confounding bias that could be ignored in the data analysis. Although *H. pylori* infection can be more accurately detected by biopsy CLO test than by the serum IgG method logically, in rare instances antral biopsies with CLO tests might not be representative of all gastric states of *H. pylori* infection. In addition, past historical data of diagnosis and treatment of *H. pylori* infection were not included in the analysis, although the enrolled cases were clinically asymptomatic. Additionally, blood insulin, gastrin levels, and proinflammatory cytokines were not measured. The study design also did not allow the identification of the pathologic mechanisms underlying the association of colorectal adenoma with metabolic syndrome and *H. pylori* infection.

The pathogenic mechanisms by which *H. pylori* exerts its malignant potential in the induction of colorectal neoplasms are not completely understood. A few studies have revealed that fecal shedding of viable *H. pylori* and its antigen occurs under certain circumstances[21,22], suggesting that *H. pylori* moves through the intestinal tract in direct contact with colonic mucosa, and could therefore locally activate colonic carcinogenesis. *H. pylori* was recently detected within colorectal carcinoma tissues[23]. The role of *H. pylori*-specific affinity for colorectal neoplasms requires further investigation. The presence of *H. pylori* might alter normal gastrointestinal flora as a consequence of progressive chronic gastritis with glandular atrophy and decreased acid production. This could further influence colorectal carcinogenesis. Persistent *H. pylori* exposure induces hypergastrinemia, which is a putative trophic factor for the large bowel mucosa. Cell proliferation and gastrin-induced genomic instability can increase the risk of DNA replication error and play a role in the development of colorectal neoplasms[24]. *H. pylori* infection might also result in direct damage to the colorectal mucosa or indirect damage to the epithelium through inflammatory responses. Contact between a repairing epithelium and endogenous or dietary carcinogens within the gut might transform the colorectal mucosa[25]. The CagA protein is the product of the cytotoxin-associated gene and is produced by cagA+ strains of *H. pylori*. It might locally activate colonic carcinogenesis through the induction of cytokine expression, including cytokines such as interleukin (IL)-8, which is associated with colorectal cancer[26]. In summary, *H. pylori* might result in local and distant interactions with colorectal mucosa and contribute to the pathogenesis of malignant transformation. However, further mechanistic studies are required.

Metabolic syndrome and its association with colorectal adenomas have been the subjects of recent study, and the pathogenic mechanisms for this potential association are still unclear. Insulin (a core contributor to metabolic syndrome) has been demonstrated to promote colorectal carcinogenesis in animal studies for more than 10 years[27,28]. It is postulated that insulin might exert proliferative effects on colonic tumor cells directly or indirectly *via* the insulin-like growth factor pathway[29]. Furthermore, increased production of proinflammatory cytokines and decreased production of anti-inflammatory adiponectin in adipocytes might be related to adenoma growth[30]. In addition, hypertriglyceridemia (a component of metabolic syndrome) might be involved in colorectal neoplasm pathogenesis. Triglycerides act as potent energy sources for cancer cell growth[31], and elevated serum triglyceride levels have been associated with increased synthesis of bile acids, which could promote large bowel carcinogenesis, as demonstrated in experimental studies[32]. Metabolic syndrome is associated with chronic inflammation, which might explain its possible association with colorectal adenoma. Adipose tissue and circulating levels of inflammatory cytokines [including tumor necrosis factor (TNF)-α and IL-6] are increased in obese and diabetic patients, and can induce several metabolic derangements characteristic of metabolic syndrome[33,34]. IL-6-induced C-reactive protein (CRP) could predict colon cancer occurrence; meanwhile, an elevated CRP level is a consistent feature of metabolic syndrome[35]. The findings indicate that chronic inflammation might be associated with colorectal carcinogenesis. In short, these evidence-base data suggest that metabolic syndrome might be a risk factor for colorectal neoplasm development.

In this study, concomitant with *H. pylori* infection and metabolic syndrome might further increase the risk of developing colorectal adenoma. The concomitant effect of metabolic syndrome and *H. pylori* might occur secondary to common inflammatory pathways of colorectal pathological mechanisms associated with metabolic syndrome and *H. pylori* infection. The inflammation-related factors of metabolic syndrome include IL-6, TNF-α, fibrinogen, and cyclooxygenase-2. The inflammation-related factors of *H. pylori* including IL-8, TNF-α, and the Cag A, Vac A, and babA2 proteins might display similar inflammatory effects attributable to the common inflammatory pathway. White blood cell counts are a risk factor of colorectal adenoma in the multivariate logistic regression analysis and might support this hypothesis of the involvement of the common inflammatory pathway. However, further investigations on the pathogenesis of this concomitant effect are necessary. Clinically, our results suggested that both *H. pylori* infection and metabolic syndrome should both be evaluated for the prevention of colorectal adenomas and carcinomas.

Studies have revealed that moderate alcohol consumption is related to increased insulin-sensitivity[36], while smoking exerted the opposite effect[37]. Other studies have suggested that both alcohol use and cigarette smoking were associated with increased risk of colorectal adenoma[38,39]. Cigarette smoking was related to colorectal adenomas in this study, although alcohol consumption was not. To clarify the association between alcohol consumption and colorectal adenoma, further studies are necessary.

In conclusion, this cross-sectional hospital-based study revealed a direct association of colorectal adenoma with *H. pylori* infection and metabolic syndrome. Furthermore, *H. pylori* infection concomitant with metabolic syndrome

might further increase the risk of colorectal adenoma. These results suggest that both *H. pylori* infection and metabolic syndrome should be considered important entities with regards to the prevention of colorectal adenoma and carcinoma. This is particularly important when a patient clinically presents with concomitant *H. pylori* infection and metabolic syndrome. The combined effects of metabolic syndrome and *H. pylori* infection should be further clarified.

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COMMENTS

Background

Colorectal cancer is one of the leading causes of cancer mortality worldwide. Colorectal adenoma is the premalignant lesion in colorectal cancer. Identification of the etiology of colorectal neoplasms might assist in the development of strategies targeted toward its prevention. Previous epidemiological studies have identified family history, dietary factors, smoking, sedentary lifestyles, and alcohol consumption as potential contributors to colorectal neoplasm development. Recently, reports revealed that *Helicobacter pylori* (*H. pylori*) infection is associated with colorectal neoplasm, and a few reports disclosed that metabolic syndrome was also associated with an increased risk of colorectal adenoma. Based on these findings, the probability of colorectal adenoma in patients with both *H. pylori* infection and metabolic syndrome was further evaluated.

Research frontiers

Colorectal cancer is an extremely common malignancy, however, the direct etiology of colorectal neoplasm is still unknown. Epidemiologically, identification of the etiology of colorectal neoplasm might assist in development of strategies targeted toward its prevention. During latest decade, *H. pylori* infection and metabolic syndrome, respectively, were identified to be associated with colorectal neoplasms and hypotheses were provided to explain the mechanisms of their relationships. However, until now, there was no study focusing on whether concomitant *H. pylori* infection with metabolic syndrome in a patient will increase his or her risk of colorectal adenoma.

Innovations and breakthroughs

This study supported the association of colorectal adenoma individually with *H. pylori* infection and metabolic syndrome. Furthermore, *H. pylori* infection concomitant with metabolic syndrome might further increase the risk of colorectal adenoma.

Applications

These results suggest that both *H. pylori* infection and metabolic syndrome should be considered important entities with regards to the prevention of colorectal adenoma and carcinoma. This is particularly important when a patient clinically presents with concomitant *H. pylori* infection and metabolic syndrome; the increased risk of developing colorectal adenomas should be more seriously considered for preventive purpose.

Peer review

This paper reports an important study that assesses the rate of metabolic syndrome, *H. pylori*, and colonic adenomas in a large population of asymptomatic individuals. The significances of these findings are discussed in relation to previous studies, and hypothesis to explain the findings are reviewed. Strengths of the study include the size of the sample, high completion rate of colonoscopy, and the use of well-established diagnostic criteria for metabolic syndrome in this population. Limitations are acknowledged by the authors and include the single centre nature of the study, select patient population, and possible false negative results of HP testing.

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BRIEF ARTICLE

Germline mutation analysis of *hPMS2* gene in Chinese families with hereditary nonpolyposis colorectal cancer

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Abstract

AIM: To study the germline mutation of *hPMS2* gene in 26 unrelated Chinese hereditary nonpolyposis colorectal cancer (HNPCC) probands and to fulfill the screening strategy for HNPCC in Chinese.

METHODS: Genomic DNA was extracted from the peripheral blood. To avoid the interference of pseudogene in detection of the remaining 11 exons (exon 1-5, 9, 11-15), long-range polymerase chain reaction (PCR) was conducted to amplify the complete coding region of *hPMS2* gene firstly. Then 1/8 of the PCR products were used as template to amplify the individual exon respectively and DNA sequencing was done. Direct DNA sequencing of the conventional PCR products of exon 6, 7, 8 and 10 of *hPMS2* gene was performed. The same analysis was made in 130 healthy persons without family histories of HNPCC to further investigate the pathological effects of the detected missense mutation.

RESULTS: One HNPCC proband fulfilled Bethesda guidelines and was found to carry the germline mutation of *hPMS2* gene, which has not been reported in Chinese HNPCC families. It was a missense mutation at c.1532C>T of exon 11. It was detected in three controls as well with an occurrence rate of 2.3% (3/130). Since it could not be found in the PMS2-single nucleotide polymorphism (SNP) database, this missense mutation is a new SNP unreported up to date. Meanwhile, 260 reported SNPs of *hPMS2* gene were detected in the 26 HNPCC probands. The 2nd and 5th exons were probably the hot SNP regions of *hPMS2* gene in Chinese HNPCC families involving 53.1% of all reported SNP.

CONCLUSION: The germline mutation of *hPMS2* gene may be rare in Chinese HNPCC families. The 2nd and 5th exons are hot SNP regions of *hPMS2* gene.

Key words: Hereditary nonpolyposis colorectal cancer; *hPMS2*; Missense mutation; Single nucleotide polymorphism; Colorectal cancer

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INTRODUCTION

Hereditary nonpolyposis colorectal cancer (HNPCC), or Lynch syndrome, is an autosomal dominantly inherited disease with cancer-susceptibility. Perhaps it is the most common cause of hereditary colorectal cancer, accounting for 5%-10% of the total colorectal cancers worldwide[1-3]. People inheriting this predisposition are at a particularly high risk of developing colorectal cancer with an early age of onset[3,4]. The affected patients always carry germline mutations in DNA mismatch repair (MMR) genes, mostly in *hMLH1, hMSH2*, and *hMSH6*[5,6]. Less commonly, mutations in other MMR genes are present. We analyzed the abnormalities of *hMSH2/hMLH1/hMSH6* genes in a series of Chinese HNPCC families fulfilling different clinical criteria. We studied germline mutation, large genomic variations of the entire coding regions of the three genes and methylation of *hMLH1* promoter in 58 Chinese HNPCC probands, in which 24 fulfilled Amsterdam criteria (AC)[7], 15 fulfilled Japanese criteria (JC)[8] and 19 met Bethesda guidelines (BG)[7]. The total detected gene abnormality rate was only 53.4% (31/58), including 29 cases of germline mutation and 2 cases of methylation of *hMLH1* promoter[9-14]. So the aberrant MMR genes other than *hMSH2/hMLH1/hMSH6* are suspected to be involved in Chinese HNPCC.

In order to accomplish our serial studies of Chinese HNPCC, we detected *hPMS2* germline mutation in 26 Chinese HNPCC families by long-range polymerase chain reaction (LR-PCR)-based sequencing in this study, and evaluated this manner in the molecular genetics screening of Chinese HNPCC.

MATERIALS AND METHODS

Materials

Twenty-six unrelated HNPCC probands registered from January 1998 to October 2005 at the Department of Abdominal Surgery in Shanghai Cancer Center were retrieved. Five of them fulfilled AC, 10 fulfilled JC and the remaining 11 fulfilled BG. Germline abnormalities of *MSH2/MLH1/MSH6* were excluded in all the 26 probands by PCR-based sequencing. Ten milliliter peripheral blood was collected from each proband for genomic DNA preparation. The peripheral blood samples of 130 healthy volunteers without any family history of hereditary disease or development of colon cancer in early age were obtained for control. The informed consents were signed by all the probands and volunteers before blood drawing. This study was approved by the Medical Ethical Committee of Shanghai Cancer Center, Fudan University. The whole procedures of the study were in accordance with the international rules and regulations.

Table 1 Primer sequences of long-range polymerase chain reaction

Primer name	Sequence (5′-3′)	Size (bp)	Exon
LRPCR1			
For	ACGTCGAAAGCAGCCAATGGGAGTT	9964	Exon 1-5
Rev	CTTCCACCTGTGCATACCACAGGCT		
LRPCR2			
For	GGTCCAGGTCTTACATGCATACTGT	9440	Exon 9
Rev	CTGACTGACATTTAGCTTGTTGACA		
LRPCR3			
For	GCGTTGATATCAATGTTACTCCAGA	8812	Exon 11,
Rev	AGTAGTCAGGGTAAAACATTCCAGT		12
LRPCR4			
For	AAAATTAGTCAGACTTGATGGTGTG	9804	Exon
Rev	CCTTCCATCTCCAAAACCAGCAAGA		13-15

DNA extraction

Genomic DNA was extracted from the peripheral blood using the QIAGEN (Hilden, Germany) DNA extraction kit and following the manufacturer's instructions. Concentrations of the genomic DNA were determined by an ultraviolet spectrophotometer (Beckman, DU640 type).

PCR amplification and DNA sequencing

LR-PCR (exon 1-5, 9, and 11-15): Since exon 1-5, 9, and 11-15 of *hPMS2* genes were severely hampered by the presence of multiple pseudogenes with highly similar sequences. LR-PCR was conducted to preferentially amplify *hPMS2* gene and avoid the interference of the pseudogenes.

Four overlapping sets of primers were designed to amplify the complete coding region of *hPMS2* gene by LR-PCR[15,16] (Table 1). The LR-PCR amplification profile is also shown in Table 1. Then 1/8 of the four LR-PCR products were used as template to amplify the 11 exons (exon 1-5, 9, 11-12 and 13-15) individually. The primer sequences are listed in Table 2.

PCR (exon 6, 7, 8 and 10): Conventional PCR was performed to detect the four exons (exon 6, 7, 8 and 10) which were seldom influenced by pseudogenes. Four sets of primers and PCR amplification profile are listed in Table 2.

DNA sequencing: The conventional PCR products were subjected to 2% agarose gel electrophoresis, while for LR-PCR products, 1% agarose was used with 9Kb as marker. After observation of clear and expected size bands, the products were purified and used as a template for sequencing reactions with BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). The sequencing primers were M13F or M13R. Automated fluorescence analysis was performed on a 3700 DNA sequence system (ABI, USA).

Bioinformatics analysis

Each result of sequencing was analyzed by DNAStar 5.08 bioanalysis software. The type of mutations and potential

Table 2 Primer sequences and polymerase chain reaction condition of individual exon of *hPMS2* gene

Exon	Primer sequence (5′-3′)	Size (bp)	AT (℃)	CN
1	M13F-ACGTCGAAAGCAGCCAATGGGAGTT M13R-CAGGTAGAAAGGAAATGCATTCAGT	475	66	28
2	M13F-ACAGTGTTGAGTCATTTCCCACAGT M13R-TTCTTAGCATAACACCTGCCTGGCA	455	66	28
3	M13F-TAGTCTGGGCTAGTAAATAGCCAGA	705	68	35
4	M13R-TATGACTTAGATTGGCAGCGAGACA			
5	M13F-CTTGATTATCTCAGAGGGATCGTCA M13R-TCTCACTGTGTTGCCCAGTCCTAAT	540	68	35
6	M13F-TGCTTCCCTTGATTTGTGCGATGAT M13R-TGAGGCAGGAGAATTGCTTGAATCT	504	67	32
7	M13F-ACCCACGAGTTTGACATTGCAGTGA M13R-GTAGAGGTTGCAGTGAGCCAAGATA	498	60	35
8	M13F-AGATTTGGAGCACAGATACCCGTGA M13R-TGCGGTAGACTTCTGTAAATGCACA	414	61	32
9	M13F-CCTTCTAAGAACATGCTGGTTGGTT M13R-ATCTCATTCCAGTCATAGCAGAGCT	279	64	45
10	M13F-AGCCCTTCCGTATTTTGTCTATTCA M13R-GCTTTAGAAGCTGTTTGTACACTGT	719	61	32
11	M13F-TCACATAAGCACGTCCTCTCACCAT M13R-GCAACAGAGCAAGACTCTGTCTCAA	1021	64	45
12	M13F-GCCAAGATTGTGCCATTGCACTGTA M13R-AGTAGATACAAGGTCTTGCTGTGTT	493	64	25
13	M13F-GTGACACTTAGCTGAGTAGTGTTGT M13R-ATGTTAGCCAGGCTGGTCTCAAACT	372	64	35
14	M13F-GGTCTGTATCTCCTGACCTCATGAT M13R-GCACGTAGCTCTCTGTGTAAAATGA	473	64	35
15	M13F-GCTGAGATCTAGAACCTAGGCTTCT M13R-ACACACGAGCGCATGCAAACATAGA	522	64	35

AT: Anneal temperature; CN: Cycle number. The sequence of M13F was 5′-GTAAAACGACGGCCAGT-3′. The sequence of M13R was 5′-AACAGCTATGACCATG-3′.

significance were determined by comparing the corresponding amino acids and proteins in the following databases (http://www.ncbi.nlm.nih.gov/; http://www.ensemble.org/homosapies; and http://www.insight-group.org).

RESULTS

Germline mutation of hPMS2 gene in HNPCC probands

Among the 26 unrelated HNPCC probands, only one (H13) was found to carry the germline mutation of *hPMS2* gene. She was a 30-year-old female BG patient. The mutation was a missense mutation at codon 511 (ACG>ATG, Thr>Met) (Figure 1). To further investigate the pathological effects of the missense mutation, we analyzed the related exon 11 in 130 controls by PCR-based sequencing. The results showed that the mutation of codon 511, consistent with the HNPCC case at c.1532C>T of exon 11 of *hPSM2* gene, was also found in three healthy controls. The occurrence rate was approximately 2.3% (3/130). It could not be found in the PMS2-SNP database (http://www.nfdht.nl; http://www.insight-group.org; and http://www.ensembl.org). Thus, the mutation at c.1532C>T of *hPSM2* gene which we detected in the HNPCC patient is an unreported new single nucleotide polymorphism (SNP).

SNP detection and analysis of hPMS2 gene

By DNA sequencing, 27 loci on the exons of *hPMS2* gene including 260 reported SNP (http://www.ensembl.org/homo_sapies) were detected in the 26 HNPCC probands. Among them, 30% (78/260) were located in the 2nd exon, 23.1% (60/260) in the 5th exon, 13.8% (36/260) in the 15th exon, 10% (26/260) in the 7th exon, and 9.2% (24/260) in the 11th exon. However, none variant was detected in the remaining exons of the 1st, 3rd, 6th, 8th, 9th and 10th. The 2nd and 5th exons were probably the hot SNP regions of *hPMS2* gene because 53.1% of the reported SNP were located in them. Distribution of the SNP of *hPMS2* gene is shown in Table 3.

DISCUSSION

HNPCC, also called Lynch syndrome, is one of the most common autosomal dominantly inherited cancer syndromes with a high risk of colorectal cancer as well as other tumors occurring in endometrium, stomach, ovary, urinary tract, pancreas, small intestine, brain and skin. People with HNPCC take about 80% risk to develop colorectal cancer in their lifetime. It accounts for 2%-15% of all colorectal cancers. Compared to sporadic colorectal cancer, HNPCC possesses its own characteristics in clinical presentations, treatment, genetic features and management of kindred[17,18]. Many countries have established the clinical diagnostic criteria for HNPCC, such as AC, JC and BG. Defects in MMR genes, mainly in *hMLH1, hMSH2* and *hMSH6* were considered to be closely related to the genetic mechanism of HNPCC. The defection would

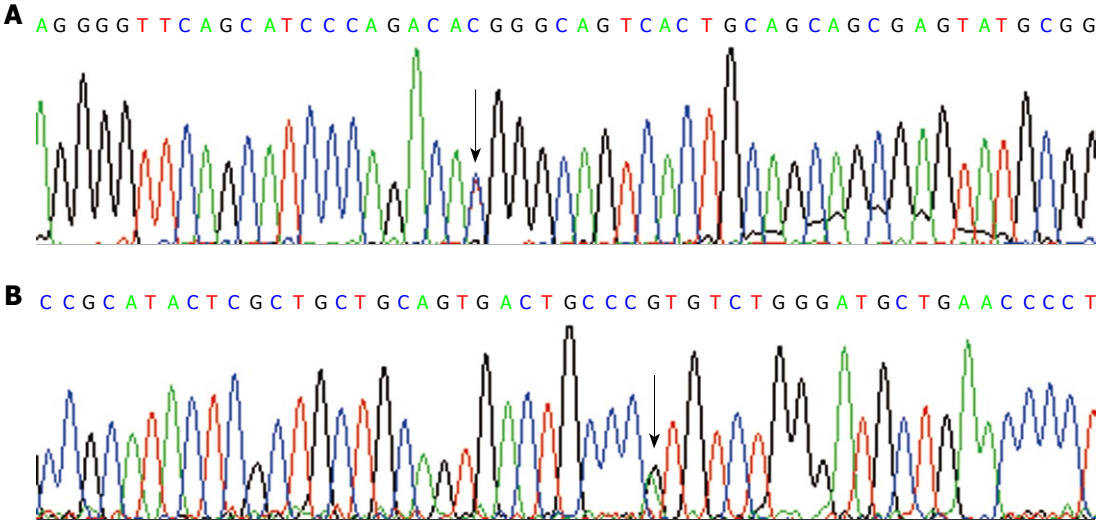

Figure 1 Missense germline mutation of exon 11 of *hPMS2* gene in the proband of H13 hereditary nonpolyposis colorectal cancer kindreds. A: The forward sequence; B: The reverse sequence. Arrow indicates the mutation site, the single basyl substitution was transversed from C to T (C>T) at the codon 511, the codon from ACG to ATG, causing the amiod acid changes from Thr>Met, the change was identified as a new single nucleotide polymorphism.

Table 3 Distribution of single nucleotide polymorphism of *hPMS2* gene in 26 probands

Exon	Nucleotide change	Amino acid change	*n*	SNP (%)
2	c.24-4C>T	-	14	78 (30)
	c.89A>C	Gln30Pro	15	
	c.117A>G	Val39Val	15	
	c.120G>A	Lys40Ly	4	
	c.121G>A	Glu41Lys	15	
	c.124T>A	Leu42Ile	8	
4	c.288C>T	Ala96Ala	8	18 (6.9)
	c.295A>C	Thr99Pro	10	
5	c.406A>G	Met136Val	10	60 (23.1)
	c.418A>G	Asn140Asp	10	
	c.429T>C	Ile143Ile	10	
	c.452G>A	Arg151His	10	
	c.478C>A	Gln160Lys	10	
	c.492C>T	Ser164Ser	10	
11	c.1408C>T	Pro470Ser	7	24 (9.2)
	c.1454C>A	Thr485Lys	11	
	c.2006+6G>A	-	7	
12	c.2007-4G>A	-	11	12 (4.6)
	c.2007-7C>T	-	1	
13	c.2253T>C	Phe751Phe	1	1 (0.4)
14	c.2324A>G	Asn775Ser	3	5 (1.9)
	c.2340C>T	Pro780Pro	2	
15	c.2466T>C	Leu822Leu	12	36 (13.8)
	c.2570G>C	Gly857Ala	2	
	c.92dupA	-	17	
	c.17G>C	-	5	

SNP: Single nucleotide polymorphism.

consequently lead to the dysfunction of MMR system, ultimately resulting in the development of neoplasm. So, detection of MMR gene mutation is the only gold criteria to make a diagnosis of HNPCC.

Within the family of MMR genes, germline mutations in the coding region of *hMSH2* and *hMLH1* could be detected in up to 45%-64% of all HNPCC families, while *hMSH6* about 10%. Previously we analyzed germline mutations and large genomic variations of the entire coding regions of *hMSH2*/*hMLH1*/*hMSH6* genes and the methylation of *hMLH1* promoter in 58 Chinese HNPCC probands, resulting in 29 germline mutations and 2 exhaustive inherited methylations of *hMLH1* promoter (excluding 3 part-methylations of *hMLH1* promoter). The total gene abnormality rate was only 53.4% (31/58). We suspected that the other MMR gene mutations might be associated with the remaining probands without *hMSH2*, *hMLH1* or *hMSH6* gene abnormalities.

The *hPMS2* gene is a member of a set of human mismatch repair genes, located on chromosome 7. It encodes the protein that plays an essential role in repairing DNA by forming an active protein complex with the MLH1 protein which interacts with MSH2 bound to mismatched bases. In 1994, Nicolaides *et al*[19] firstly found the germline mutation of *hPMS2* gene in a HNPCC patient. Since then, more and more data have proved that *hPMS2* germline mutation is involved in the development of HNPCC. In some reports, it could be detected in as high as 62% of HNPCC probands[20]. The *hPMS2* gene was suggested as the first candidate gene for testing germline mutations in HNPCC families in which *hMSH2*, *hMLH1*and *hMSH6* aberrant was excluded. However, genetic testing for germline mutation of *hPMS2* gene was technically challenging because they were severely hampered by a large family of highly homologous pseudogenes located on the same chromosome as the true *hPMS2*, such as *PMS2CL*. They shared similar sequences to *hPMS2* but had no functions. Data from literature indicated that the exon 6 to 8 and exon 10 of *hPMS2* could be easily screened by direct sequencing of genomic DNA without interference of pseudogenes. But detection of exon 1-5, 9 and exon 11-15 was complicated due to the interference of *PMS2*CL. LR-PCR was recommended as a useful method to preferentially identify *hPMS2* but not the pseudogenes. In this study, we used LR-PCR to investigate the germline mutation of *hPMS2* gene in those

HNPCC probands who did not carry *hMLH1/hMSH2/hMSH6* germline mutations investigated by the previous studies. Four overlapping sets of primers were designed to amplify the complete coding region of *hPMS2* gene by LR-PCR firstly. Then, exon-specific amplifications from the LR-PCR products were performed to obtain a clear sequence with no evidence of pseudogene contamination. We only found one missense mutation in 26 probands, which has not been reported in Chinese HNPCC families. This mutation could also be detected in the 130 control persons with an occurrence rate of about 2.3%. Since it could not be found in the PMS2-SNP database (http://www.nfdht.nl; http://www.insight-group.org; and http://www.ensembl.org), the mutation at c.1532C>T of *hPMS2* gene in our HNPCC case was an unreported new single nucleotide polymorphism (SNP). Our results showed that the germline mutation of *hPMS2* gene was probably a rare event in Chinese HNPCC, even in those probands without *hMLH1/hMSH2/hMSH6* mutations. It was consistent with the results of some other studies[21]. Interestingly, another mutation was found in the same nucleotide, c1532_1533 delCGinsAC, causing the amiod changes from Thr to Asn (http://www.insight-group.org). So, the exon 11 may be a hot SNP or mutation region of *hPMS2* gene.

The frequency of germline mutation in *hPMS2* gene was reported to be up to 62% if patients whose tumor tissues lacked protein expression of *hPMS2* or had MSI-H features, were selected[22]. Among the HNPCC families with monoallelic mutation in *hPMS2*, 65.5% were complied with BG. Recently, Niessen *et al*[23] identified 4 patients with pathogenic mutation of *hPMS2* among 97 patients with suspected Lynch syndrome who carried no germline mutation in *hMLH1*, *hMSH2* or *hMSH6*. All these 4 patients fulfilled BG and their corresponding tumor cells showed MSI-H and loss of expression of *hPMS2*. Clendenning *et al*[24] reported that a kind of frame-shift mutation of *hPMS2* occurred in 12 ostensibly unrelated Lynch syndrome patients with 20% being the deleterious mutation. However, those families with pathogenic mutation did not have significantly high incidence of Lynch syndrome associated malignant tumors, indicating that the germline mutation of *hPMS2* and occurrence of HNPCC were not concurrent sometimes. The patient with *hPMS2* gene mutation in our group also met the requirements of BG. By reviewing the family history of our mutation positive patient, we found that in her first-degree relatives, three suffered from colorectal cancer but diagnosed at age over 60 years, not in accordance with the typical feature of HNPCC. Although we are not so certain about this, the non-classical presentation of her family history, to some extent, represents the phenomenon of separation of HNPCC occurrence and *hPMS2* gene mutation.

At the same time, we detected the reported SNP in these 26 probands and found some interesting results. Most of the SNP (21/27) were in the exons and 12 were non-synonymous coding SNP(cSNP). Since these non-synonymous cSNP can induce the change of amino acid and the relationship between cSNP and pathogenesis of HNPCC still remains unclear, whether they are involved in the development of HNPCC and HNPCC related tumors needs to be further investigated.

In conclusion, the germline mutation of *hPMS2* gene is rare in the probands of Chinese HNPCC families. Since the testing of *hPMS2* gene mutation is costly and complicated, it may be not reasonable to be included in the screening strategy of Chinese HNPCC. However, the frequency of SNP of *hPMS2* gene is high and further studies are needed to identify its relationship with HNPCC.

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COMMENTS

Background

Germline mutations in mismatched repair genes can lead to hereditary nonpolyposis colorectal cancer (HNPCC). Previously, the authors had analyzed the abnormalities of *hMSH2*/*hMLH1*/*hMSH6* genes in a serious of Chinese HNPCC families and the total abnormality rate was only 53.4% (31/58). So the aberrant MMR genes such as *hPMS2* were suspected to be involved in Chinese HNPCC.

Research frontiers

HNPCC or Lynch syndrome, is an autosomal dominantly inherited disease with cancer-susceptibility. The testing of *hPMS2* gene mutation is costly and complicated, it may be not reasonable to be included in the screening strategy of Chinese HNPCC. However, the frequency of single nucleotide polymorphism (SNP) of *hPMS2* gene is high and further studies are needed to identify its relationship with HNPCC.

Innovations and breakthroughs

One HNPCC proband was found to carry the germline mutation of *hPMS2* gene. It was a new unreported coding SNP, which could also be detected in the control with an occurrence rate of 2.3% (3/130).

Applications

Germline mutations in genes can be used to diagnose early HNPCC and enrich the databases about HNPCC and SNP.

Terminology

HNPCC is an abbreviation of hereditary nonpolyposis colorectal cancer. Germline mutations are the mutations in genomic DNA.

Peer review

This is an interesting article which deals with a remarkably rare germline mutation, namely PMS2, which is important in the etiology of Lynch syndrome (HNPCC). Their science appears to be sound.

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CASE REPORT

Poorly differentiated endocrine carcinoma of the pancreas responded to gemcitabine: Case report

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Abstract

Poorly differentiated endocrine carcinoma (PDEC) of the pancreas is a rare and aggressive tumor. First-line treatment is commonly a combination of etoposide and cisplatin, but there is no consensus regarding further treatment recommendations. In this report, we describe a case of pancreatic PDEC treated with gemcitabine as third-line chemotherapy. A 62-year-old man with pancreatic PDEC was administered etoposide plus cisplatin as first-line treatment; he then received irinotecan for tumor relapse. However, because irinotecan induced ileus in this patient, we chose gemcitabine as third-line chemotherapy. After two cycles of gemcitabine (1000 mg/m^2 on days 1, 8 and 15 every 4 wk), a partial tumor response was noted by computed tomography (approximately 68% reduction in tumor size). Our patient survived for 15 mo after diagnosis. This is a rare case of unresectable pancreatic PDEC, which showed a partial response to gemcitabine after the failure of two other regimens. Gemcitabine could be an effective treatment option for pancreatic PDEC that is resistant to other treatments.

Key words: Poorly differentiated endocrine carcinoma; Pancreatic endocrine tumor; Gemcitabine; Chemotherapy

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INTRODUCTION

Pancreatic endocrine tumors (PETs) are rare neoplasms with an annual incidence of less than 1 per 100 000 people[1-6]. These tumors account for less than 1%-2% of all pancreatic neoplasms[1,7]. Poorly differentiated endocrine

carcinoma (PDEC) of the pancreas is characterized by aggressive tumor biology and poor prognosis. The biological behavior of PDEC is similar to that of small-cell lung cancer (SCLC), and metastatic pancreatic PDECs are often treated with the chemotherapy regimens that are used to treat SCLC. The combination of etoposide and cisplatin has been widely used to treat pancreatic PDEC because no promising chemotherapy regimens have been reported for this disease. Effective second- or later-line chemotherapy is still uncertain. Gemcitabine is an active agent against untreated and recurrent SCLC. In this report, we describe a case of pancreatic PDEC treated with gemcitabine as third-line chemotherapy.

CASE REPORT

A 62-year-old man with Crohn's disease had previously received treatment at a different hospital. In July 2007, his serum carcinoembryonic antigen (CEA) level was found to be elevated. A contrast-enhanced computed tomography (CT) scan of the patient's abdomen showed a tumor in the head of the pancreas and enlarged para-aortic lymph nodes. In September 2007, he underwent exploratory laparotomy, during which peritoneal dissemination was observed, and hence, a biopsy of the para-aortic lymph nodes was conducted. Based on the histological findings, small cell carcinoma of the pancreas was diagnosed.

Because the tumor was unresectable at the time of diagnosis, the patient was treated with a combination of etoposide and cisplatin as first-line chemotherapy in October 2007. The chemotherapeutic response was deemed to be partial, until multiple bone metastases to the skull, vertebrae, and pelvis were detected using CT after five cycles of chemotherapy. The patient was next administered irinotecan monotherapy as second-line chemotherapy, which started in March 2008. Irinotecan was stopped after one cycle because ileus occurred. He was referred to our hospital for further treatment in July 2008.

The patient had no family history of cancer, and the results of a physical examination were unremarkable. The laboratory findings were hemoglobin 11.5 g/dL (normal 14.0-17.0 g/dL), γ-glutamyl transpeptidase 113 IU/L (normal, 10-47 IU/L), glucose 136 mg/dL (normal, 69-104 mg/dL), CEA 12.8 ng/mL (normal, < 4.0 ng/mL), carbohydrate antigen 19-9 14 U/mL (normal, < 37 U/mL), neuron-specific enolase (NSE) 36.2 ng/mL (normal, < 10.0 ng/mL), and pro-gastrin-releasing peptide (pro-GRP) 338 pg/mL (normal, < 46 pg/mL). A contrast-enhanced CT scan of his abdomen revealed a low-density mass, 7.5 cm in diameter, in the head of the pancreas, as well as enlarged para-aortic lymph nodes at the time of admission. The pancreatic tumor did not show contrast enhancement (Figure 1A). A CT scan of his chest did not show any primary or metastatic pulmonary tumors. We reviewed an excised biopsy specimen of a para-aortic lymph node obtained at the previous hospital. Histological examination of the specimen showed small to intermediate-sized cells with a high nuclear-cytoplasmic ratio and frequent mitosis, and partial necrosis. Immunohistochemical staining revealed that these cells were strongly positive for NSE, CD56, and keratin; weakly positive for chromogranin A; and negative for vimentin, leukocyte common antigen, S-100, and CD99 (Figure 2). On the basis of the pathological findings, the para-aortic lymphadenopathy was determined to be caused by metastasis of PDEC. Therefore, pancreatic PDEC with para-aortic lymph nodes and bone metastases was diagnosed.

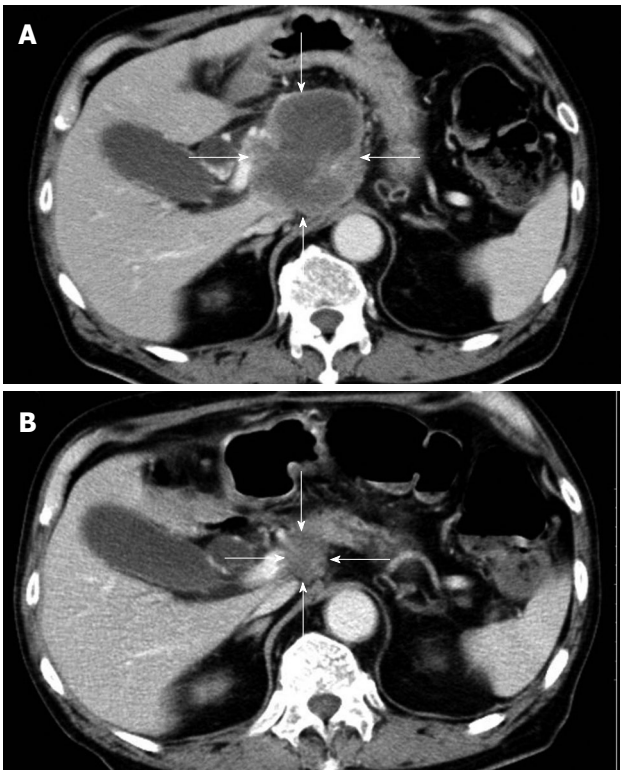

Figure 1 Contrast-enhanced computed tomography scan of the abdomen. A: There was a low-density mass, 7.5 cm in diameter, in the head of the pancreas at the time of admission. The pancreatic tumor (arrows) did not show contrast enhancement; B: A follow-up computed tomography scan showed that the pancreatic mass had reduced to 2.0 cm in diameter. The tumor (arrows) had markedly regressed 4 mo after starting chemotherapy with gemcitabine.

We chose gemcitabine as third-line chemotherapy. Starting in July 2008, the patient received 1000 mg/m^2 gemcitabine on days l, 8 and 15 every 4 wk.

After two cycles of gemcitabine, a CT scan of his abdomen showed regression of the pancreatic tumor (from 7.5 cm to 2.4 cm in diameter), and his serum NSE and pro-GRP levels had decreased to within the normal range. The chemotherapeutic response was deemed to be a partial response. After four cycles of gemcitabine, an abdominal CT scan showed a pancreatic mass that was 2.0 cm in diameter (Figure 1B). In November 2008, after day 15 of the fifth cycle, the patient requested that the therapy be stopped because of general fatigue. He died of multiple organ failure in December 2008.

DISCUSSION

Pancreatic PDEC is a rare neoplasm. Recently, Bettini *et al*[8]

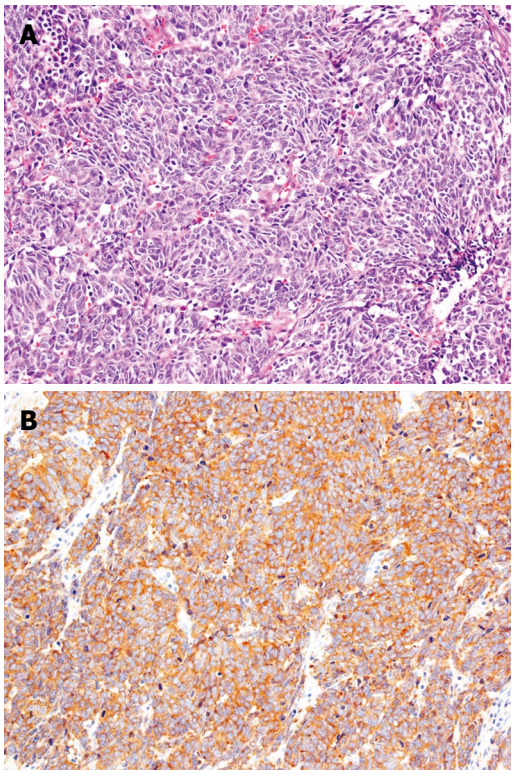

Figure 2 Histopathological findings. A: The excised para-aortic lymph node showed small to intermediate-sized cells with a high nuclear-cytoplasmic ratio. (HE stain, original magnification, × 200); B: Immunostaining for neuron-specific enolase was positive in the cytoplasm of many tumor cells (original magnification, × 200).

have reported that PDEC was diagnosed in 17 (9.4%) of 180 patients with non-functioning pancreatic endocrine tumors. PDEC is characterized by aggressive tumor biology and poor prognosis. Bettini *et al*[8] also have reported that all patients with PDEC died within 3.5 years after diagnosis (median, 11.8 mo), and that only 23.5% of the tumors were resectable at the time of diagnosis. Our patient survived for 15 mo after diagnosis. His survival time was longer than the median survival time that was reported by Bettini *et al*[8].

The standard treatment for advanced pancreatic PDEC has not yet been established. The initial approach to treatment of pancreatic PDEC is to attempt curative resection. However, liver and lymph node metastases are present in 32.5% and 59.5% of patients at the time of diagnosis[9]. Therefore, curative surgical resection cannot be achieved in most patients, and effective medical treatment to control metastatic lesions is urgently required. Systemic chemotherapy is proposed for patients with inoperable pancreatic PDEC, and adequate organ function and performance status; however, a standard chemotherapeutic regimen has not been established. In our patient, the tumor was inoperable owing to the presence of peritoneal dissemination and para-aortic lymph node metastases, and hence, systemic chemotherapy was administered to the patient.

The biological behavior of PDEC is similar to that of SCLC, and metastatic pancreatic PDECs are often treated with the same chemotherapy regimens that are used to treat SCLCs. Combination chemotherapy with etoposide and cisplatin, one of the standard regimens for SCLC, is commonly used to treat pancreatic PDEC.

Moertel *et al*[10] have reported that etoposide plus cisplatin produced good therapeutic results in patients with anaplastic neuroendocrine carcinoma (which has been defined as PDEC according to the recent WHO classification[11]), with an overall regression rate of 67% and a median regression duration of 8 mo [10]. Other investigators have reported similar results, with a median duration of response of 7-9 mo in patients with poorly differentiated endocrine tumors[12,13].

Since the report of Moertel *et al*[10], the combination of etoposide and cisplatin has been considered to be the reference treatment for PDEC; however, confirmatory studies have not been performed because of the rarity of PDEC. If this first-line chemotherapy fails to treat pancreatic PDEC, there is no consensus regarding further treatment recommendations. Irinotecan plus cisplatin is one of the standard regimens for extensive-stage SCLC[14]. In our case, the patient had been administered irinotecan monotherapy as second-line treatment before he was referred to our hospital. However, this therapy had been discontinued because ileus occurred after one cycle.

Several newer anticancer drugs, including paclitaxel[15], topotecan[16] and gemcitabine[17], have shown little activity as single agents against neuroendocrine tumors (NETs). Gemcitabine is a nucleoside analog with structural similarities to cytarabine and is widely used in the treatment of advanced pancreatic adenocarcinoma[18]. In a phase Ⅱ trial of gemcitabine for the treatment of metastatic NETs, Kulke *et al*[17] have reported that, although the treatment was well tolerated, no radiological responses were observed, 65% of the patients (n = 18) experienced disease stabilization, and that the median overall survival was less than 1 year. However, their study included various histological subtypes of NETs, and only two of the 18 patients had poorly differentiated NETs. Thus, the efficacy of gemcitabine for poorly differentiated NET of the pancreas remains unclear.

Gemcitabine is an active agent against untreated and recurrent SCLC[19-21]. The response rate to gemcitabine was reported to be 27% in patients with previously untreated SCLC[19]. In patients with previously refractory or recurrent SCLC treated with at least one chemotherapeutic regimen, gemcitabine resulted in response rates of 6%-17%[20,21].

We believe that gemcitabine is a reasonable treatment option for pancreatic PDEC, and we chose gemcitabine as third-line chemotherapy. After two cycles of gemcitabine, the pancreatic tumor showed marked regression, which resulted in a partial response. Gemcitabine has shown good efficacy as third-line chemotherapy for refractory pancreatic PDEC. The prognosis of pancreatic PDEC is extremely poor because of its highly aggressive behavior, and hence, effective second- and later-line treatments are important for improving prognosis. In light of this, gem-

citabine could be an effective treatment option for pancreatic PDEC that is resistant to other treatment.

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Reply to "Application of contrast-enhanced intraoperative ultrasonography in the decision-making about hepatocellular carcinoma operation"

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Abstract

The use of contrast-enhanced intraoperative ultrasound for hepatocellular carcinoma has been already proposed as a novel technique to stage the disease during surgical resection. In the herein presented "letter to the editor", the authors underline some important points, which have been raised following the paper by Wu *et al*.

Key words: Hepatocellular carcinoma; Liver surgery; Contrast-enhanced intraoperative ultrasound; Cirrhosis

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TO THE EDITOR

The recent paper by Wu *et al*[1] entitled "Application of contrast-enhanced intraoperative ultrasonography in the decision-making about hepatocellular carcinoma operation" published in the January issue of *World Journal of Gastroenterology* reports some experiences of the authors in contrast-enhanced intraoperative ultrasound (CEIOUS) for hepatocellular carcinoma (HCC). This paper raises a couple of questions that, we believe, need to be asked about.

First and foremost, the authors did not mention any of the previously performed and published studies on the same topic on the application of CEIOUS for HCC[2-7]. Such studies not only represent the first pioneer investigations on CEIOUS, but up to now they are the cornerstones of this new intraoperative imaging modality, which needs to be confirmed or confuted by further studies performed by other groups. In this sense, Wu *et al*[1] have lost this opportunity.

Second, it is unclear to the readers how the authors defined a lesion as malignant based on the CEIOUS findings. This is a pivotal point. Yet, CEIOUS for HCC requires a kind of classification to interpret its findings in order to make the correct diagnosis. In particular in case of cirrhotic liver, where the finding of multiple subcentimetric nodules is common, the typical arterial phase might not be very clear because some of those nodules are high-grade dysplastic nodules or early HCC with no anticipated standard contrast enhancement. Indeed, we proposed a classification that, we believe, could help in this sense, even if it probably requires some refinements[8].

Third, the reported value of specificity for CEIOUS is very high compared with that for intraoperative ultra-

sound (IOUS) and contrast-enhanced magnetic resonance imaging (CEMRI). The impression is that the authors calculated the specificity by adding the value of CEMRI and IOUS. When CEIOUS was performed on the same population of patients who had CEMRI and IOUS, its results in terms of sensitivity and specificity might be biased by the previous radiological findings. Only a true blind performance of different diagnostic methods might allow a true comparison in terms of diagnostic accuracy.

Finally, we thank that Wu *et al*[1], because our group, developed and supported the study of CEIOUS performed many years ago, both for HCC[2,4-6] and for colorectal liver metastases[9]. Thus, any new study on the same topic further sustains its use.

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Meetings

Events Calendar 2010

January 25-26
Tamilnadu, India
International Conference on Medical Negligence and Litigation in Medical Practice

January 25-29
Waikoloa, HI, United States
Selected Topics in Internal Medicine

January 26-27
Dubai, United Arab Emirates
2nd Middle East Gastroenterology Conference

January 28-30
Hong Kong, China
The 1st International Congress on Abdominal Obesity

February 11-13
Fort Lauderdale, FL, United States
21th Annual International Colorectal Disease Symposium

February 26-28
Carolina, United States
First Symposium of GI Oncology at The Caribbean

March 04-06
Bethesda, MD, United States
8th International Symposium on Targeted Anticancer Therapies

March 05-07
Peshawar, Pakistan
26th Pakistan Society of Gastroenterology & Endoscopy Meeting

March 09-12
Brussels, Belgium
30th International Symposium on Intensive Care and Emergency Medicine

March 12-14
Bhubaneswar, India
18th Annual Meeting of Indian National Association for Study of the Liver

March 23-26
Cairo, Egypt
14th Pan Arab Conference on Diabetes PACD14

March 25-28
Beijing, China
The 20th Conference of the Asian Pacific Association for the Study of the Liver

March 27-28
San Diego, California, United States
25th Annual New Treatments in Chronic Liver Disease

April 07-09
Dubai, United Arab Emirates
The 6th Emirates Gastroenterology and Hepatology Conference, EGHC 2010

April 14-17
Landover, Maryland, United States
12th World Congress of Endoscopic Surgery

April 14-18
Vienna, Austria
The International Liver Congress™ 2010

April 28-May 01
Dubrovnik, Croatia
3rd Central European Congress of surgery and the 5th Croatian Congress of Surgery

May 01-05
New Orleans, LA, United States
Digestive Disease Week Annual Meeting

May 06-08
Munich, Germany
The Power of Programming: International Conference on Developmental Origins of Health and Disease

May 15-19
Minneapolis, MN, United States
American Society of Colon and Rectal Surgeons Annual Meeting

June 04-06
Chicago, IL, United States
American Society of Clinical Oncologists Annual Meeting

June 09-12
Singapore, Singapore
13th International Conference on Emergency Medicine

June 14
Kosice, Slovakia
Gastro-intestinal Models in the Research of Probiotics and Prebiotics-Scientific Symposium

June 16-19
Hong Kong, China
ILTS: International Liver Transplantation Society ILTS Annual International Congress

June 20-23
Mannheim, Germany
16th World Congress for Bronchoesophagology-WCBE

June 25-29
Orlando, FL, United States
70th ADA Diabetes Scientific Sessions

August 28-31
Boston, Massachusetts, United States
10th OESO World Congress on Diseases of the Oesophagus 2010

September 10-12
Montreal, Canada
International Liver Association's Fourth Annual Conference

September 11-12
La Jolla, CA, United States
New Advances in Inflammatory Bowel Disease

September 12-15
Boston, MA, United States
ICAAC: Interscience Conference on Antimicrobial Agents and Chemotherapy Annual Meeting

September 16-18
Prague, Czech Republic
Prague Hepatology Meeting 2010

September 23-26
Prague, Czech Republic
The 1st World Congress on Controversies in Gastroenterology & Liver Diseases

October 07-09
Belgrade, Serbia
The 7th Biannual International Symposium of Society of Coloproctology

October 15-20
San Antonio, TX, United States
ACG 2010: American College of Gastroenterology Annual Scienitfic Meeting

October 23-27
Barcelona, Spain
18th United European Gastroenterology Week

October 29-November 02
Boston, Massachusetts, United States
The Liver Meeting® 2010--AASLD's 61st Annual Meeting

November 13-14
San Francisco, CA, United States
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Acknowledgments

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2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Write as mean ± SD or mean ± SE.

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