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PERFORATED PEPTIC ULCER IN ESTONIA: EPIDEMIOLOGY, RISK FACTORS AND RELATIONS WITH *HELICOBACTER PYLORI*

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LIST OF ORIGINAL PUBLICATIONS THAT THE PRESENT THESIS IS BASED ON

I Sillakivi T, Lang A, Soplepmann J, Tein A, Peetsalu A. Incidence of perforated peptic ulcer correlated with suicide rate in Estonia in 1981–2000. Proceedings of the Eurosurgery 2002. Monduzzi Editore. Lisbon (Portugal), June 5–7, 2002: 199–204.

II Sillakivi T, Lang A, Tein A, Peetsalu A. Evaluation of risk factors for mortality in surgically treated perforated peptic ulcer. Hepato-Gastroenterol 2000; 47: 1765–1768.

III Sillakivi T, Yang Q, Peetsalu A, Ohmann C. Perforated peptic ulcer: Is there a difference between Eastern Europe and Germany? Langenbeck's Arch Surg 2000; 385: 344–349.

IV Sillakivi T, Aro H, Ustav M, Peetsalu M, Peetsalu A, Mikelsaar M. Diversity of *Helicobacter pylori* genotypes among Estonian and Russian patients with perforated peptic ulcer, living in Southern Estonia. FEMS Microbiol Letters 2001; 195: 29–33.

V Sillakivi T, Peetsalu M, Mikelsaar M, Peetsalu A. An Attempt for *Helicobacter pylori* Eradication with Intravenous Clarithromycin in Perforated Peptic Ulcer Patients. Scand J Gastroenterol 2001; 36: 1119–1120.

VI Andreson H, Lõivukene K, Sillakivi T, Maaroos H-I, Ustav M, Peetsalu A, Mikelsaar M. Association of *cagA* and *vacA* genotypes of *Helicobacter pylori* with gastric diseases in Estonia. J Clin Microbiol 2002; 40: 298–300.

VII Sillakivi T, Peetsalu A. Perforeerunud peptilise haavandi riskifaktorid. Eesti Arst 2000; 12: 718–722.

VIII Sillakivi T, Peetsalu M, Mikelsaar M, Peetsalu A. *Helicobacter pylori* eradikatsioon perforeerunud peptilise haavandiga haigetel. Eesti Arst 2001; 1: 8–11.

ABBREVATIONS

PPU	perforated peptic ulcer
DU	duodenal ulcer
GU	gastric ulcer
PUD	peptic ulcer disease
CG	chronic gastritis
H. pylori	*Helicobacter pylori*
NSAID	Nonsteroidal Antiinflammatory Drugs
PCR	Polymerase Chain Reaction
vacA	vacuolating cytotoxin gene A
cagA	cytotoxin associated gene A
PPI	proton pump inhibitor
CI	confidence interval
OR	Odds ratio

1. INTRODUCTION

Peptic ulcer is naturally a chronic disease with frequent exacerbations which can last 20–30 years (Penston, 1990). The pathology of gastric ulcer was first described by Creuveilhier in 1835 (Creuveilhier, 1835). Having changed from a rare disease in the 19th century to one of the most common conditions in the human by the middle of the 20th century, peptic ulcer has shown the tendency to decline in incidence in developed countries since the 1950s (Coggon *et al.,* 1981). At the same time, significant geographical and regional differences have been established in the prevalence of peptic ulcer (May, 1985; Weir *et al.,* 1968; Kurata *et al.,* 1984).

Perforated peptic ulcer (PPU), a serious and potentially fatal complication of peptic ulcer disease, was first described already in 1843 (Crisp, 1843). In about 10% of population, peptic ulcer disease develops during lifetime (Langman, 1979), while the incidence of (PPU) between 2.3 and 10.0 has been reported from developed countries in recent decades (Aeberhard *et al.,* 1990; Hermansson *et al.,* 1997; Mäkelä *et al.,* 1992). In recent decades, PPU incidence has decreased in young patients and males but has increased in elderly patients and females (Svanes, 2000). Use of NSAIDs among PPU patients, as well as the rate of concomitant diseases and complications have increased (Aeberhard *et al.,* 1990; Svanes *et al.,* 1993; Bliss *et al.,* 1991; Bulut *et al.,* 1996; Suter, 1995).

In Estonia, according to the reports of the Ministry of Social Affairs of Estonia, the number of emergency operations for PPU per year has increased about 2.5-fold since 1991. The reasons for such an increase have not yet been evaluated. They could be related to changes in the factors involved in the pathogenesis and course of ulcer disease, as well as to the extensive use of ulcerogenic medicines in community, changes in the policies of the medical treatment of ulcer, changes in the circulating strains of *H. pylori*, etc. Moreover, it is important to assess whether the changes involve groups of the working population as well as to identify the role that socio-economic factors could play in Estonia where fast changes have taken place in the political situation.

Treatment policies for PPU in developed countries have changed as well. Easy nondefinitive operations, not altering the pathogenetic mechanisms of ulcer disease, have gained prevalence (Roher *et al.,* 1996; Druart *et al.,* 1997). Such changes in tactics are based mainly on availability of new powerful medicines and introduction of *H. pylori* treatment in management. At the same time, unlike another ulcer disease complication, ulcer haemorrhage (Sonnenberg *et al.,* 1999; Laine, 1995), in PPU the long-term results of this treatment tactics have not been adequately published. Nor has the role of H. pylori itself been unambiguously identified in ulcer perforation. (Reinbach *et al.,* 1993; Sebastian *et al.,* 1995; Matsukura *et al.,* 1997). In addition, a large number of studies are focused on the evaluation of different *H. pylori* strains and the relationships of the virulence markers of bacteria with development of different

gastric diseases (Rudi *et al.,* 1998; Strobel *et al.,* 1998; van Doorn *et al.,* 1998). However, these correlations have not yet been unambiguously clarified (Maeda *et al.,* 1998; Atherton *et al.,* 1997).

Problems of peptic ulcer and gastric surgery have already been studied for several decades in Estonia and at the Tartu University Clinic of Surgery (Sarv, 1968; Sibul *et al.,* 1968; Truve *et al.,* 1973; Lutsuver, 1978; Peetsalu *et al.,* 1991; Väli *et al.,* 1994; Vardja *et al.,* 1996; Soplepmann *et al.,* 1997; Peetsalu *et al.,* 1998). The present investigation continued this work focusing on ulcer complication, PPU, whose incidence seemed to have been increased significantly in our patients since the last decade. The study dealt mainly with the following aspects: epidemiology of PPU in the last two decades, possible risk factors for PPU, evaluation of the effect of different surgical treatment strategies on outcome and relationship of PPU with *H. pylori*, including evaluation of postoperative eradication regimens in our patients.

The purpose of the investigations was also to support the elaboration of evidence based guidelines for PPU treatment in Estonia by providing corresponding regional and national data.

2. REVIEW OF THE LITERATURE

2.1. Epidemiology of PPU

A large number of international data indicate that the epidemiology of PPU has undergone considerable changes in developed countries in recent decades. Subjects born after the turn of the 20th century have carried a high risk for PPU throughout their lives; the increasing ulcer risk among the elderly today is related to the aging of these high risk cohorts; in younger generations the incidence is decreasing (Svanes, 2000). Mean age of patients at the time of perforation has risen by 10–20 years (Svanes *et al.,* 1993; Hermansson *et al.,* 1997). Being previously a disease affecting predominantly men (male:female ratio about 10:1), PPU is now increasingly affecting elderly women in developed countries (male:female ratio about 1.5:1) (Svanes *et al.,* 1993). Use of non-steroidal anti-inflammatory drugs among PPU patients, as well as the rate of concomitant diseases and complications have increased considerably (Aeberhard *et al.,* 1990; Hermansson *et al.,* 1997; Bliss *et al.,* 1991; Bulut *et al.,* 1996; Suter, 1995). The increasing proportion of women among PPU patients is suggested to be influenced by a rising prescription rate for NSAIDs among elderly women and the higher life expectancy of women (Mäkelä *et al.,* 1992; Walt *et al.,* 1986).

The location of ulcer has also changed. In Crisp's series (Crisp, 1843) gastric ulcer perforations accounted for about 70% of all perforations among which half were close to the cardia. In the middle of the 20th century most perforations were located in the duodenum (Jamieson, 1955). At the end of the 20th century there was described a certain increasing tendency for praepyloric and gastric ulcer perforations (Svanes *et al.,* 1993; Aeberhard *et al.,* 1990; Tonnesen *et al.,* 2001). Patients with perforated gastric ulcers are often elderly (Svanes *et al.,* 1993; Lanng *et al.,* 1988), predominantly females (Lanng *et al.,* 1988).

The rate of mortality has not declined during recent decades despite of the progress of medical care. Mortality is reported to vary between 5–31% (Aeberhard *et al.,* 1990; Svanes *et al.,* 1993; Bulut *et al.,* 1996; Suter, 1995; Khorsovani *et al.,* 1994; Hermansson *et al.,* 1997) in different studies. Today most deaths occur when patients have concomitant serious illness; the slight increase in death and complication rates during recent decades can be ascribed largely to older and generally more ill patients (Svanes *et al.,* 1993). Mortality rates differ significantly in young patients and in those over 70 years (Hermansson *et al.,* 1997; Irvin, 1989), being nearly 0% in patients under age 50 years and over 30 % in patients over 70 years.

Majority of peptic ulcer perforations now occur in previously undiagnosed ulcers, being often the first manifestation of ulcer disease (Gunshefski *et al.,* 1990). Also, increase in treatment delay seems to be part of a general trend, which has a certain impact on nonimproving mortality (Svanes *et al.,* 1993;

Bodner *et al.,* 1990; Horowitz *et al.,* 1989). Using multifactor analysis Svanes et all found that unchanging mortality rates are at least partly masked by the fact that increasingly older patients are undergoing surgery and the advances in anesthesia and in peri- and postoperative care could be seen if different age groups were analysed (Svanes *et al.,* 1989).

At the same time, no information of the above described trends among the Estonian population is available, and it is unclear whether similar changes have occurred in Eastern Europe.

2.2. Risk factors related to PPU development

Smoking seems to be one of the main risk factors for PPU, especially in persons aged under 70 (Svanes *et al.,* 1997; Reinbach *et al.,* 1993; Svanes, 2000). Smoking has been shown to increase the risk for ulcer perforation about tenfold both among men and women. (Svanes *et al.,* 1997; Andersen *et al.,* 2001).

Use of NSAIDs is another well proven important risk factor for ulcer perforation, which increases the risk five- to eightfold (Rodriquez *et al.,* 1994; Svanes *et al.*, 1996; Bliss *et al.*, 1991).

PPU has been associated with social stress (Lam *et al.,* 1995; Jamieson, 1955; Cleave, 1962), and suicide rate has been pointed out as an indirect stress parameter (Schneider, 1973). Suicide rate has been increasing rapidly in Estonia starting from 1991 (Wasserman *et al.,* 1994), however, it has not yet been evaluated whether the PPU and suicide rates were statistically correlated.

Development of perforation in peptic ulcer patients has also been related to the following risk factors: alcohol consumption (Sharma *et al.,* 1997; Andersen *et al.,* 2001), use of crack-cocaine (Sharma *et al.,* 1997) and partial type of hunger, Ramadan (Donderici *et al.,* 1994). The influence of birth cohort trends in PPU prevalence has been suggested as well, i.e. the disease risk follows particular birth cohorts (Svanes *et al.,* 1995). Subjects born at the beginning of the 20th century carried a high risk for PPU, while subjects born before the turn of the century, or more recent birth cohorts showed lower incidence (Svanes, 2000).

The importance of the main factor in the pathogenesis of ulcer disease, *H. pylori* infection, is not clear in perforated peptic ulcer. In contrast with opinions in favour of the crucial role of *H. pylori* (Sebastian *et al.,* 1995; Tokunaga *et al.,* 1998; Ng *et al.,* 1997; Mihmanli *et al.,* 1998) several researchers have opposed this idea (Reinbach *et al.,* 1993; Matsukura *et al.,* 1997; Chowdhary *et al.,* 1998; Beales, 1998), maintaining that PPU represents even a specific subgroup of peptic ulcer disease (Beales, 1998). The divergence of studies may be partly attributed to the differences in the methods used in *H. pylori* detection, or to the ethnic diversity of patients (Tokunaga *et al.,* 1998). Also, differences in

H. pylori strains and host factors can serve as important factors (Matsukura *et al.,* 1997).

Prevention of ulcer perforation can be achieved through avoidance of smoking; this is an aspect that may play a greater role in ulcer surgery, compared even with *H. pylori* eradication (Svanes, 2000).

There is lack of data about these risk factors in Estonian PPU patients, although it was reported, that the prevalence of *H. pylori* in whole Estonian population (up to 87%) is higher than in most European countries (Maaroos, 1995).

2.3. Treatment strategies in PPU and risk factors for mortality

The surgical treatment of perforated peptic ulcer dates from the year 1880, when Mikulicz sutured a perforated gastric ulcer for the first time (Mikulicz, 1885). Conservative management of PPU (intravenous fluid resuscitation, antibiotics, nasogastric suction and acid reducing pharmacotherapy) as an alternative to surgery in selected patients was first advocated by Tylor in 1946 (Tylor, 1946). Although some studies with good results were published (Tylor, 1946; Crofts *et al.,* 1989) this strategy was never generally accepted, as conservative treatment includes some risk for safety of diagnosis and difficulties with continuous clinical monitoring as well as significant disadvantages in the case of delayed laparotomy (Hölscher *et al.,* 2001). Today most centres prefer surgical methods in treatment of PPU.

In recent decades, since the introduction of the H2-receptor antagonists in 1978, a substantial decrease has occurred in the number of elective operations for peptic ulcer, whereas the number of emergency operations due to ulcer complications (including PPU) has remained the same (Aeberhard *et al.,* 1990; Paimela *et al.,* 1991; Welch *et al.,* 1986; Towfight *et al.,* 2002). With a new understanding of peptic ulcer pathogenesis and the advent of new powerful medicines, simple operations as ulcer suturation, including laparoscopic management, have been increasingly advocated for optimal surgical treatment of PPU in recent years (Cocks, 1992; Al-Assi *et al.,* 1994; Lau *et al.,* 1995; Roher *et al.,* 1996; Druart *et al.,* 1997; De Boer, 1997; Tran *et al.,* 2002). However, there exists a certain controversy in this issue. Numerous data give evidence of better long-term results after definitive operation: recurrence rate is lower and the patient's self-estimation of his/her condition is higher (Jordan *et al.,* 1995; Robles *et al.,* 1995; Peetsalu *et al.,* 1994; Tsugawa *et al.,* 2001). At the same time, the advantage of *H. pylori* eradication for PPU patients, which has been demonstrated to reduce recurrent ulceration and rebleeding in peptic ulcer hemorrhage (Sonnenberg *et al.,* 1999; Laine *et al.,* 1995) and which is the main factor bringing about such fast changes in surgical treatment, is not yet clear.

Furthermore, the increasing proportion of *H. pylori* negative patients with ulcer complications, especially among elderly patients (Laine *et al.,* 1998; Maher *et al.,* 1997), and the fact that eradication rates do not reach 100% despite any efforts, indicates that the role of surgery cannot be fully abandoned in these patients. Thus Donovan et al proposed considering *H. pylori* negative patients as candidates for definitive ulcer-curative operation (Donovan *et al.,* 1998).

Several studies discussing the safety of and risk factors for definitive surgery in an emergency situation have established that definitive surgery in an emergency situation is as safe as nondefinitive surgery (Gunshefski *et al.,* 1990; Boey *et al.,* 1982; Evans *et al.,* 1997), even in high risk patients (Hamby *et al.,* 1993; Schein *et al.,* 1990).

Higher age, delayed treatment, presence of concomitant diseases, presence of shock on admission, immunocompromised state, low blood hemoglobin level, low albumin concentration, leucocyte count less than 9,500/mm3 and location of ulcer in the gastric corpus have been pointed out as the risk factors for complications and mortality (Suter, 1995; Khorsovani *et al.,* 1994; Svanes *et al.,* 1989; Evans *et al.,* 1997; Wakayama *et al.,* 1994; McIntosh *et al.,* 1996; So *et al.,* 2000; Chan *et al.,* 2000; Tsugawa *et al.,* 2001). Mortality risk increases rapidly if multiple independent risk factors occur in a person and reach up to 100% when three or more of the above mentioned risk factors coexist (Chan *et al.,* 2000; Boey *et al.,* 1987).

There has not been found any correlation between mortality and smoking or alcohol use in PPU patients (McIntosh *et al.,* 1996; Bodner *et al.,* 1990; Horowitz *et al.,* 1989).

Nor have the factors determining outcome after surgical treatment in Estonian PPU patients been analysed before.

2.4. *Helicobacter pylori* and PPU

In 1982, a spiral-shaped bacterium occurring in the human stomach (Marshall, 1983; Warren, 1983), known nowadays as *Helicobacter pylori,* was isolated for the first time. The discovery of *H. pylori* infection has transformed our understanding of the patogenesis of peptic ulcer disease (McColl, 1997). *H. pylori* is probably one of the commonest chronic bacterial infections found in humans which is distributed in the whole world and is associated with several gastric diseases as chronic gastritis, peptic ulcer, gastric cancer and MALT (*mucosal-associated lymphoid tissues)* gastric lymphoma. *H. pylori* infection is more frequent among the population of developing countries (80–90%) compared with the population of developed countries (50%) (Glupczynski, 1996). The infection is mostly acquired in childhood and is often lifelong. It is thought that spread of *H. pylori* infection is related to socio-economic situation rather than to racial belonging (Malaty *et al.,* 1992).

Despite the widespread distribution of *H. pylori* in the world, the pathway of dissemination of the infection is yet unclear. In most cases, the spread is considered to be fecal-oral, primarily in developing countries where an important role is evidently played by contamination of water (Klein *et al.,* 1991; Lindkvist, 1999).

In majority of persons the course of *H. pylori* infection is asymptomatic. This circumstance raises a very important problem, namely, if the difference between different host organisms in response to *H. pylori* infection is the case, or if only certain *H. pylori* strains possess virulent markers needed for damaging the gastric mucosa. These relationships have not been clarified until now.

2.4.1. Prevalence of *H.pylori* infection in PPU patients

The prevalence of *H. pylori* infection nowadays is about 50% in western populations (The EUROGAST Study Group, 1993), ranging between about 20% in 20-years-olds and more than 70% in those over 70 years (Cullen *et al.,* 1993; Veldhuyzen van Zanten *et al.,* 1994; Kosunen *et al.,* 1989). In Estonia *H. pylori* infection was found in 78–87% of the adult population studied and, according to immunological studies, the *H. pylori* was positive in 87% of Estonian adults and in 56% of schoolchildren (Maaroos, 1995; Vorobjova *et al.,* 1994; Vorobjova *et al.,* 1998). At the same time, its prevalence in PPU patients is described to vary between 50 and 96% (Reinbach *et al.,* 1993; Sebastian *et al.,* 1995; Matsukura *et al.,* 1997, Tran *et al.,* 2002). The prevalence of *H. pylori* in Estonian PPU patients has not yet been studied.

2.4.2. *H. pylori* genotypes

To date, several products of *H. pylori* genes like *vacA* and its subtypes and *cagA*, acting as disease associated pathogenic factors in infected persons, have been identified (Atherton *et al.,* 1995; Atherton *et al.,* 1997; Censini *et al.,* 1996).

The *vacA* gene is present in all *H. pylori* strains, but only approximately 50% of them can produce cytotoxin inducing vacuolisation of gastric epithelial cells (Cover *et al.,* 1992; Atherton *et al.,* 1995), the cause of this being actually different intensities of toxin production (Reyrat *et al.,* 1999).

In addition, *vacA* alleles occur with different genotype variations (mosaic pattern) where three different families of signal sequences (s1a, s1b and s2) and two different families of middle-region alleles (m1 and m2) were initially distinguished (Atherton *et al.,* 1995). By now, a number of different middle-region types have been additionally found (m1a, m1T and m1Tm2, m1b and

m1b-m2) (Pan *et al.,* 1998; Wang *et al.,* 1998) and one more subtype of the signal sequence, s1c, has been added (van Doorn *et al.,* 1998).

About 60% of *H. pylori* strains possess the *cagA* gene, among which nearly all produce CagA protein (Tummuru *et al.,* 1993). CagA is thought to be associated with the cytotoxic activity of a *H. pylori* strain (Cover *et al.,* 1990; Tummuru *et al.,* 1993).

The virulent cytotoxin VacA and CagA producing strains are described to be more common among patients with peptic ulcer and gastric cancer (Atherton *et al.,* 1997; Cover *et al.,* 1994). However, in Asia the high prevalence of CagA positivity has hampered its use as a virulent marker of *H. pylori* strains (Shimoyama *et al.,* 1997; Pan *et al.,* 1997). A study performed in Japan found no significant difference in the prevalence of the virulence factors between the *H. pylori* strains isolated from patients with peptic ulcers and chronic gastritis (Tokumaru *et al.,* 1999).

According to published studies (Atherton *et al.,* 1997; Cover *et al.,* 1994; van Doorn *et al.,* 1998) the *vacA* s1a/m1 subtype is considered more cytotoxic and more related to development of peptic ulcer disease compared with the s1a/m2 and s2/m2 *vacA* genotypes.

However, the relationship between development of different gastric diseases and the virulence markers of *H. pylori* strains has not been investigated in a large number of different populations.

A key feature of *H. pylori* is the enormous genomic diversity of the strains distributed over the globe. It has even been suggested that many individuals appear to be infected by a unique strain (Owen, 1998).

In contrast, a particular geographic pattern of different *H. pylori* genotypes seems to be characteristic of different countries (Ito *et al.,* 1997; Rudi *et al.,* 1998). In Western populations, gastric atrophy, duodenal ulceration, intestinal metaplasia and gastric carcinoma are more common among patients infected with *cagA* positive (and *vacA* s1 subtype) than among patients infected with *cagA* negative strains (Atherton *et al.,* 1995 and 1997). Yet in China and Japan, *H. pylori* strains possessing or lacking the *cagA* gene were equally frequent among diseased and control patients (Ito *et al.,* 1997; Pan *et al.,* 1997). Recently, it was shown that in East Asia and in the Western countries, distinct variants of *H. pylori cagA* genes were associated with particular *vacA* subtypes (Van Doorn et al, 1999).

However, the association of particular *H. pylori* genotypes with their virulence for the host is not yet definitely proved. Particularly, the incidence of peptic ulcer disease was not reflected in the frequency of different *H. pylori* lineages among different races as Polynesians and European New Zealanders (Campbell *et al.,* 1997). Obviously, more studies are needed to compare the differences in the *H. pylori* genome in particular ethnic groups with the same underlying disease, living in the same geographic region. Relevant literature data are not sufficient and such investigations have not been performed in Estonian PPU patients at all.

2.4.3. Eradication of *H. pylori*

The evidence of the role of *H. pylori* in the pathogenesis of peptic ulcer and its eradication therapy have considerably changed the whole peptic ulcer management policy. According to the Maastricht Consensus Report 1 from 1996 (European Helicobacter Pylori Study Group: Current European Concepts on the Management of Helicobacter Pylori Infection. The Maastricht Consensus Report. 1996) and Maastricht Consensus Report 2 from 2000 (The European *Helicobacter pylori* Study Group, 2000) *H. pylori* eradication is nowadays strongly recommended for all ulcer patients with confirmed infection. Eradication rates in peroral triple or quadruple regimens exceed 80–90 % in most Western studies (Tytgat, 1998; Wermeille *et al.,* 1998). At the same time, almost no data are available about the results of such strategy in PPU patients, as such patients are mainly excluded from studies. Only preliminary data on PPU patients suggest, that *H. pylori* eradication after simple ulcer suturation prevents ulcer recurrence in the subsequent 1-year period (Ng *et al.,* 2000), however, published data or long term results on such patients have yet been scanty.

Long-term studies conducted in Estonia (follow-up of patients up to 14 years after operation) have shown that nearly all gastric mucosa in DU patients is affected by *H. pylori* infection; that vagotomy as an effective method of ulcer treatment does not eliminate *H. pylori* colonization in the stomach, although it suppresses temporarily acid production; that the number of recurrent ulcers increases steadily with time from operation (Peetsalu *et al.,* 1991; Peetsalu *et al.,* 1998).

Therefore, it appears rational to eliminate *H. pylori* infection in PPU patients by using antibacterial treatment immediately after operation in order to decrease ulcer recurrence. According to available literature data, such tactics have been attempted, and the results have been promising. Friess and coauthors (Friess *et al.,* 1992) followed eradication of *H. pylori* in proximal vagotomy patients after a single course of perioperative preventive intravenous antibiotics (Meslocillin + Metronidazol). After a 3-month follow-up, eradication of *H. pylori* was achieved in 85% of patients. In other reports *H. pylori* eradication rates in nonoperated peptic ulcer bleeding patients ranged from 44% (Sheu *et al.,* 1999) to 87% (Romero *et al.,* 2000) and were 93% in patients with gastroduodenal ulcer disease (Adamek *et al.,* 1994), with application of intravenous regimens. Hence we supposed that it might be advantageous to use clarithromycin treatment for eradication of *H. pylori* infection in our PPU patients immediately after operation. Evidently, such treatment tactics cannot be equally effective in different countries due to the different antibiotic susceptibility of *H. pylori* strains. As clarithromycin had not been introduced in Estonia before our study, one could expect no resistance of *H. pylori* to this compound. Our aim was to find a convenient, efficient and possibly short postoperative *H. pylori* eradication regimen for PPU patients.

The efficiency of different *H. pylori* eradication regimens for Estonian PPU patients has not yet been evaluated.

2.4.4. Failure of *H. pylori* eradication

There is clear evidence that *H. pylori* treatment fails in 5% to 20% of the time in any regimen used (Tytgat, 1998; Graham *et al.,* 1992). Failure of eradication may be related to many reasons or of their combinations.

Noncompliance, often related to side effects, is one of the most important reasons for ineffective therapy as pointed out by many researchers (Graham *et al.,* 1992; Tylor *et al.,* 1997; Labenz *et al.,* 1995; Qasim *et al.,* 2002). Graham and his colleagues found a 30% reduction in efficacy in patients who took less than 60% of their medications (Graham *et al.,* 1992).

The efficacy of the treatment of *H. pylori* infection can be reduced by occurrence of primary or acquired resistance to various drugs, especially metronidazole (Axon, 1991). In the resistance of metronidazole a significant difference was found between the developed and the developing countries (Megraud, 1997; Iovene *et al.,* 1999). The rate of resistance to clarithromycin, which is another widely used antibiotic for *H. pylori* eradication, varies from 0–15% in Europe and is correlated with the extent of the use of the drug in a community (Megraud, 1997; Iovene *et al.,* 1999; Megraud, 1998). In Estonian patients studied, 46% (26 of 56) of *H. pylori* strains were metronidazole resistant while all strains were clarithoromycin–sensitive (Lõivukene *et al.,* 2000). In contrast, *H. pylori* does not appear to develop significant resistance to amoxicillin (Glupczinski *et al.,* 1995) and consumption of antibiotics in population is not correlated with the acquired resistance of *H. pylori* (Lõivukene *et al.,* 2002). At the same time, in vitro determined drug resistance is not directly correlated with treatment success (Peitz *et al.,* 1999).

Although poor compliance and bacterial resistance are important factors in determining treatment success, they cannot explain all failures, suggesting that other factors must be involved (Wermeille *et al.,* 2002).

In certain conditions as deficit of nutrients and excess of antibiotics and oxygen, etc., *H. pylori* can form coccoid forms (Donelli *et al.,* 1998; Nakamura *et al.,* 2000). The coccoid forms of *H. pylori* can occur in three states: dead-degenerative, viable but not culturable and viable-culturable. Coccoid forms play obviously an important role in successful spread of the infection as well as in treatment failure (Andersen *et al.,* 2000). During a one-week treatment *H. pylori* shifts from the antrum to the corpus and the fundus (Logan *et al.,* 1995). It is possible that the so-called dormant forms occur extraventricularly as well (Atherton *et al.,* 1995).

H. pylori strains are characterised by genetic variability. The patient may be colonised by more than one strain (Prewett *et al.,* 1992), which means that there may occur competition for localisation and nutrients between different strains.

In case a treatment resistant strain is accidentally shaded by a treatment sensitive strain and remains thus nonisolated, further treatment may prove complicated (Prewett *et al., 1992).*

Literature provides some evidence that cytotoxic *H. pylori cagA* positive sl/m1 or sl/m2 strains respond better to *H. pylori* eradication therapy compared with less cytotoxic *cagA* negative s2/m2 strains (van Doorn *et al.,* 2000; Treiber *et al.,* 2002). However, this has not been evaluated in complicated gastric diseases as PPU.

Therapy with PPI prior to eradication has been shown to reduce the efficiency of eradication, while pre-treatment with H2 blockers does not influence it (Labenz *et al.,* 1995).

Eradication has been reported to be impaired also by smoking (Haruma *et al.,* 1999; Labenz *et al.,* 1995; Treiber *et al.,* 2002; Perri *et al.,* 2001), localization of the ulcer in the duodenum (Labenz *et al.,* 1995), female sex (Moayyedi *et al.,* 1997), younger age of the patient and use of too short regimens (5–7 days) (Treiber *et al.,* 2002 Labenz *et al.,* 1995).

Different eradication regimens are not equally effective in different regions and countries. Unfortunately, good or excellent results obtained from randomized controlled trials do not predict the same rate of effectiveness in general practice (Perri *et al.,* 1999). Therefore, the choice of effective eradication therapy, suitable for patients with PPU, has not yet been elaborated.

3. AIMS OF THE STUDY

1. To examine the epidemiology of PPU in a defined area in the southern part of Estonia, Tartu county, during the 20-year period from 1981 to 2000.
2. To compare the characteristics of PPU patients and treatment policies, complications and outcome of PPU disease in Eastern Europe (including Estonia) with respective data from Western countries (Germany).
3. To assess the possible risk factors for high PPU incidence in Estonian patients.
4. To evaluate the outcome and possible risk factors for mortality in PPU patients with a special reference to the type of operation performed.
5. To determine the prevalence of *H. pylori* and to compare its genomic variation in patients with PPU, living in the same area of Estonia but belonging to different nationalities.
6. To compare the distribution of well-known virulence markers (*cagA* and *vacA*) for *H. pylori* strains isolated from southern Estonian patients suffering from chronic gastritis (CG), peptic ulcer disease (PUD) and perforated peptic ulcer (PPU).
7. To evaluate different postoperative *H. pylori* eradication regimens in PPU patients.

4. PATIENTS AND METHODS

4.1. Subjects

In the presented studies altogether 780 patients were included; 504 PPU patients admitted to the Clinic of Surgery of Tartu University Clinics were involved, 129 of them in prospective studies. There occurred some overlapping between the study groups, as in six different studies from 6 to 426 of the PPU patients were included (**Table 1**). In addition, 134 PPU patients from 11 East European centres and 37 PPU patients from Germany were analysed. Apart from PPU patients, also 105 nonoperated patients, among them 36 chronic gastritis patients and 69 uncomplicated peptic ulcer patients from Southern Estonia, were included in the studies.

Table 1. Catchment of PPU patients in the studies (summarised).

Study type Period of data collection	Site	Total No. of patients (males/females)	Goals of the study (pts. included in part. analysis)	Publications (No)
1. Retrospective 1981–2000	Tartu Univ.Clinic	426 (331/95)	Epidemiology of PPU (426)	Proceedings of Eurosurg 2002 (I)
2. Retrospective 1978–1997	Tartu Univ. Clinic	394 (319/75)	Risk factors for mortality in PPU (386)	Hepato-gastro-enterol(II)
3. Prospective	Multicentre	207 (165/42)	1) East European vs German PPU	
a) Copernicus 1997	12 East European Hospitals	170* (141/29)	patients (207) 2) risk factors for complications and	Langenbeck's Arch Surg (III)
b) MEDWIS 1994–1996	11 German Hospitals	37 (24/13)	mortality in PPU (206)	
4. Prospective 1997–1999	Tartu Univ. Clinic	129 (96/33)	1) possible risk factors for high PPU incidence (129)	Eesti Arst (VII)
			2) *H.pylori* strains in PPU (51)	FEMS Microbiol Lett (IV)
			3) eradication of *H.pylori* in PPU (49/6)	Eesti Arst (VIII) Scand J GE (V)
5. Prospective 1995–2000	Tartu Univ. Clinic	51 PPU 69 PUD 36 CG	*H.pylori* strains in PPU, PUD and CG patients (156)	J Clin Microbiol (VI)

*includes also 36 PPU patients from Tartu Univ.Clinic

4.1.1. Retrospective study of the epidemiology of PPU in Tartu county in 1981–2000.

All 426 patients with PPU, admitted to the Tartu University Clinic from the defined area of Tartu county between 1981 and 2000, were included in a retrospective study. The Tartu University Clinic is the only medical institution which serves patients with PPU in the area. Tartu county accounts for about one tenth of the Estonian population. The population of Tartu county was relatively stable during the study period. The estimated mean population was 156,993 (standard error ±896 and range 151,010–163,113) throughout the 20-year study period according to the Statistical Yearbooks published by the Statistical Office of Estonia. No considerable changes took place in the age or sex distribution of the population in this period. Incidence of PPU was calculated per 100,000 inhabitants per year.

Primary data for the identification of patients were obtained from the computerised database of the hospital according to discharge diagnosis. Further, case histories and, when needed, also the hospital's discharge registry and logbooks of the operating theatre were studied. No changes took place in disease classification during the studied period.

The following information was drawn: patient's age and sex, previously confirmed (endoscopically or on X-ray) ulcer disease, presence of concomitant diseases, ulcer location and outcome. Sex and age related analyses were performed on the basis of all patients; the rest of the analyses were carried out including only patients with available data. It was impossible to evaluate the data on smoking, use of non-steroidal anti- inflammatory drugs and ulcer medication in the retrospective part of the study on the basis of these documents.

The diagnosis of PPU was confirmed in 426 patients. In 398 patients it was confirmed on operation and in four patients on autopsy. Four conservatively managed patients were also included in whom all of the following three criteria were documented: typical clinical signs, pneumoperitoneum on X-ray and endoscopically verified ulcer disease.

To compare the study periods, the patients were divided into two groups according to different decades: period I (1981–90) and period II (1991–2000).

The correlation between the annual incidences of PPU in Tartu county and the suicide rates in Estonia for 1981–2000 was evaluated using the Pearson correlation test. The data of the suicide rates in Estonia were drawn from the official database of the Swedish-Estonian Institute of Suicidology.

4.1.2. Retrospective study evaluating the risk factors for mortality in perforated peptic ulcer patients in Tartu county in 1978–97

In another retrospective study conducted for evaluating the risk factors for mortality in PPU patients, using the same methodology, all 394 patients admitted to the Tartu University Clinic in 1978–97 were included. The following data were recorded from case histories and entered into the database: patients' age and sex, previous ulcer history, concomitant diseases, shock on admission (systolic blood pressure <100 mmHg), treatment delay, ulcer location, type of operation, complications and outcome. Evaluation of the possible risk factors for mortality was carried out on 386 out of 394 (i.e. only surgically treated) patients using univariate analysis. The analysis involving age, sex and operative method was performed on all patients; the other parameters were evaluated on available patients. Nonoperatively treated patients were excluded from risk stratification. Further multivariate logistic regression was performed to analyse the mortality risk factors. Only five significant factors from univariate analysis were included in the model.

4.1.3. Multicentral international prospective studies conducted in 1994–96 (MEDWIS A 70,Germany) and in 1997 (Copernicus AAP 555, East Europe). Comparison of East European and German PPU patients, evaluation of the risk factors for complications and mortality

Sixteen centres from nine countries participated in an Eastern European prospective multicentre study of acute abdominal pain (COPERNICUS). Patient recruitment took place between 1 January and 31 October 1997, including ultimately 170 patients from 12 centres (incl.36 patients from Tartu) with the final diagnosis of PPU. Ten centres from Germany and one from Austria participated in a similar study, conducted between October 1994 and March 1996 (MEDWIS A 70), the final diagnosis of PPU was confirmed in 37 persons. In both studies a structured standardised medical history was drawn up for all patients and a physical examination was performed according to international standards. These clinical findings together with the data of laboratory investigations, ultrasound, x-ray, diagnosis, operative procedure and outcome were documented prospectively using a computer program. For documentation, a software package with multilingual program versions was developed. For all parameters the same standardised definitions were used in both studies. The data collected at the centres were transmitted anonymously to the central study secretariat in Düsseldorf and were examined with respect to completeness and quality. Evaluation of the possible risk factors for complications and mortality was carried out on 206 out of 207 patients. One patient dropped out due to insufficient data.

4.1.4. Prospective study conducted at Tartu University Clinic in 1997–1999: possible reasons for high PPU incidence in Estonian patients, *H. pylori* strains and postoperative eradication therapy in PPU patients

In a prospective study conducted between 1 January 1997 and 31 December 1999, all 129 patients with the final diagnosis of PPU, admitted to Tartu University Clinic, were included and data were collected according to special protocols.

On the basis of this database, the possible risk factors for ongoing high PPU incidence in our patients were evaluated.

The presence of *H. pylori* infection prior to eradication was assessed histologically in nonconsecutive 96 patients of 129 and using PCR technique in 51 patients of 129; in PCR analysis only the patients whose both parents could be identified as being of the same nationality (Estonian or Russian) were included.

Postoperative eradication of *H. pylori* was attempted in 51 nonconsecutive patients of 129, among them two discontinued treatment due to side effects (diarrhoea).

4.1.5. Prospective study conducted Tartu University in 1995–2000: *H. pylori* in patients with different gastric diseases

In a study carried out at Tartu University Clinics between 1995 and 2000, altogether 156 patients with 3 different gastric diseases were collected. In addition to previously described 51 PPU patients (whose both parents were identified as being of the same nationality), also 69 PUD (peptic ulcer disease) patients and 36 CG (chronic gastritis) patients referred to the Tartu University outpatient clinic for upper gastrointestinal endoscopy from southern Estonia were analysed. In the case of CG and PUD, *H. pylori* strains were isolated from gastric mucosal biopsy samples obtained by endoscopy. Endoscopic diagnoses were made in 69 cases of PUD (DU in 61 cases and GU in 8 cases). In 36 cases without PUD on endoscopy, histologically CG was diagnosed according to the Sydney classification (Misiewicz *et al.,* 1990). From the 51 PPU patients, gastric specimens of the antral mucosa were obtained for PCR analysis intraoperatively or postoperatively by panendoscopy.

4.2. Definitions and terminology: PPU, classification of ulcer locations, ulcerogenic drugs, operations and mortality

The diagnosis of PPU was confirmed on operation or autopsy; conservatively managed patients were also included if all of the following three criteria were documented: typical clinical signs, pneumoperitoneum on X-ray and endoscopically verified ulcer disease prior to perforation.

The ulcers were classified into gastric (GU) and duodenal ulcers (DU) according to the site, while DU comprised prepyloric (within 0.5–2.0 cm from the pyloric ring), pyloric and bulbar ulcers, as described earlier by Horowitz (Horowitz *et al.,* 1989).

Ulcerogenic drugs included NSAIDs and glycocorticoids.

Nondefinitive operations included ulcer excision or suturation. Definitive operations included truncal vagotomy combined with pyloroplasty or antrumectomy, selective proximal vagotomy with ulcer excision or suturation, and partial gastrectomy.

Mortality was assessed during the time the patient remained in hospital, irrespective of the 30-day period.

4.3. Determination of *H. pylori* infection in the gastric mucosa

4.3.1. Use of histological method

Biopsy specimens were taken intraoperatively through the ulcer perforation using biopsy forceps. Two-three months after the patient finished *H. pylori* eradication therapy specimens were collected on panendoscopy. In both cases two specimens from the gastric corpus and two from the antrum were collected and fixed in neutral buffered formalin solution and embedded in paraffin. *H. pylori* was later determined semiquantitatively after staining the tissue sections by the modified Giemsa method as in earlier studies (Peetsalu *et al.*, 1991). A blind examination of all specimens was performed, two from the corpus and two from the antrum. The amount of *H. pylori* was estimated semiquantitatively (Stolte *et al.,* 1989). If *H. pylori* colonisation was patchy, the grade was classified according to the most pronounced colonisation. When evaluating the presence of bacteria prior to eradication, or the results of *H. pylori* eradication, the patient was considered *H. pylori* positive in all cases if the bacteria were found at least in one specimen of the four. The histological evaluation of the specimens was performed by Margot Peetsalu within cooperative research.

4.3.2. Use of PCR method

For isolation of *H. pylori,* the biopsy samples from patients with CG and PUD were placed into Stuart Transport Medium and taken to the laboratory within 2h. The gastric specimens of the antral mucosa of the 53 PPU patients were taken intraoperatively or postoperatively on panendoscopy: these biopsy samples were placed directly into the lysis buffer (200 mM Tris-HCl [pH 8.0],

25 mM EDTA, 300 mM NaCl, 1.2% sodium dodecyl sulfate) and stored at -20^0C.

Further the samples were processed and DNA was extracted as described in detail in publications IV and VI (Sillakivi *et al.*, 2001; Andreson *et al.*, 2002). For analysis of the s and m regions of *vacA* and for detection of the *cagA* gene, the primers shown in the Table (Table 1 in publication IV, FEMS Microbiol Letters, 2001;195: page 30) were used. The PCR analyses were performed by Helena Andreson within cooperative research.

4.4. Eradication regimens for *H. pylori*

Six different regimens were used postoperatively. There were five peroral 7-days regimens (groups A–E), beginning from the 7^{th}–10^{th} postoperative day (i.e. outpatients), and one intravenous 5-day regimen (group F), beginning from the first postoperatiive day (i.e. inpatients).

The use of regimens was changed stepwise with the aim to find a simple, effective regimen. In 1997 we started with a classical combination (amoxicillin, metronidazole, omeprazole) favoured in Estonia at that time. After unsuccessful results clarithromycin monotherapies were initiated in 1998. However, as the results were still unsatisfactory, the next combination, advocated by the Estonian Gastroenterologists' Guideline, combining amoxicillin, clarithromycin and omeprazole, was started in 1998.

For nonvagotomized patients two "classical" 7-day peroral triple therapies were used:

- Group A — amoxicillin-rat. 1000mg b.i.d + metronidazole 500mg q.i.d. + omeprazole (Losec) 20mg b.i.d.
- Group B — amoxicillin-rat. 1000mg b.i.d + clarithromycin (Klacid) 500mg b.i.d. + omeprazole (Losec) 20mg b.i.d.

The rest of the regimens were modified.

For vagotomized patients the modifications of these previously described regimens, excluding proton pump inhibitor (PPI) (Losec), were used with the aim to evaluate the hypothesis that vagotomy replaces PPI in such cases:

- Group C — amoxicillin-rat. 1000mg b.i.d + metronidazole 500mg q.i.d.
- Group D — amoxicillin-rat. 1000mg b.i.d + clarithromycin (Klacid) 500mg b.i.d.

Clarithromycin monotherapy was used both in vagotomized and nonvagotomized patients in 1998 when this medicine had not been used in Estonia previously, and we could expect no *H.pylori* resistance to this drug:

- Group E — clarithromycin (Klacid) 500mg b.i.d.
- Group F — clarithromycin (Klacid) 500mg b.i.d. (intravenous!).

4.5. Statistical analysis

The mean values are presented as the mean ± standard error of the mean and were compared with the use of Student's t-Test. Absolute numbers were compared by the means of chi-square test with continuity correction. The association between PPU incidences and suicide rates was evaluated using the Pearson correlation test. In the Copernicus and MEDWIS studies, medians with the percentiles of 25–75 were calculated for age. For continuous variables the Mann Whitney-test was employed. Multivariate analysis with stepwise logistic regression (BMDP LR) involved variables that proved to be significant in univariate analysis using the default values of the program (enter limit: 0.1, remove limit: 0.15). Differences were considered statistically significant for p values less than 0.05.

5. RESULTS

5.1. Epidemiology of PPU in Tartu county in 1981–2000 (publication I)

The incidence of PPU remained between 4 and 12 (mean 7.4±0.9) per 100,000 inhabitants per year in period I (**Figure 1 in publication I,** Proceedings of the Eurosurgery 2002. Monduzzi Editore. Lisbon (Portugal), June 5–7, 2002: page 201). In period II there occurred a sharp increase in the incidence (up to 26), which did not drop below 14 (mean 20.0±1.3) ($p<0.001$).

The Pearson correlation test revealed a statistically significant correlation between the annual incidences of PPU and the suicide rates in the period 1981–2000 ($r=0.633$, $p=0.0027$).

An increasing tendency of PPU incidence was similar both for male and female patients, while the female:male ratio (1:4.1 in period I and 1:3.3 in period II) did not reveal any significant changes (p=ns).

Mean age was almost the same, 47.4±1.7 years in 1981–90 and 46.4±1.1 years in 1991–2000 (p=ns). The mean age of women, 62.2±2.0 years, exceeded significantly that of men, 42.3±0.9 ($p<0.001$). Mean age for GU perforations was 56.9±2.3 years and for DU perforations 45.0±1.0 years ($p<0.001$).

GU perforations accounted for 11.1% (13/113) and 11.9% (37/304) of all perforations in periods I and II, respectively (p=ns). For female patients GU perforations constituted 18.5% (17/92) of all perforations, exceeding the respective figure for males, 10.5% (34/325) ($p<0.05$). In patients aged ≥65 years the proportion of GU perforations was higher, 22.1% (17/77), compared with patients aged <65 years, 10.0% (34/340) ($p<0.01$).

Of the patients 30.4% (112/369) had a history of confirmed ulcer disease before perforation and 23.4% (94/402) had concomitant diseases.

Twenty-five patients (5.9%) of 426 with PPU died during 1981–2000. Mortality in PPU patients remained almost the same, 6.0% (7/117) in period I and 5.8% (18/309) in period II (p=ns). Mortality for females was 15.8% versus 3.0% for males ($p<0.001$). However, it revealed no significant difference when different age groups were compared: 24.2% (8/33) for males and 27.1% (13/48) for females, both aged ≥65, and 0.7% (2/298) for males and 4.3% (2/47) for females, both aged <65. The mean age of patients who died, 74.2±2.5 years, exceeded significantly that of patients who survived, 45.0±0.9 years ($p<0.001$).

5.2. Comparison of East European and German PPU patients: epidemiology, treatment strategies and outcome (publication III)

Among the 170 East European patients with PPU, male patients dominated over females with a ratio of 4.9:1, while among the 37 German patients, the male to

female ratio was only 1.8:1 (**Table 1 in publication III,** Langenbeck's Arch Surg, 2000; 385: page 345) ($p<0.05$). Median age was 43.0 (33.0–55.3) years for the East European patients and 49.0 (32.0–73.0) years for the German patients (p=n.s.); however, it was 53.0 (40.5–71.5) years for female patients in the East European study and 73.0 (55.0–82.0) years for female patients in the German study ($p<0.05$). There was a significant difference in ulcer location between the studies, gastric ulcer perforations accounting for 23.5% (40 of 170) in the East European study and 54.1% (20 of 37) in the German study ($p<0.001$). Comparison of the two studies revealed no significant differences in delay on admission, shock on admission, previous dyspepsia, previously diagnosed ulcer or use of ulcerogenic drugs.

The distribution of operations performed in PPU patients is shown in **Table** (**Table 2 in publication III,** Langenbeck's Arch Surg, 2000; 385: page 346). In the German study only 5 (16.1%) patients were operated using a definitive method, while in the East European study 67 (41.1%) of the patients underwent a definitive operation ($p<0.01$). In the German study no vagotomies were performed for PPU.

There were no significant differences in the overall complication rate, but 35.1% (13 of 37) of the German patients had general complications compared with 12.4% (21 of 169) of the East European patients (**Table 3 in publication No III,** Langenbeck's Arch Surg, 2000; 385: page 346).

In the East European study 2.4% (4 of 170) of the patients and in the German study 13.5% (5 of 37) of the patients died ($p<0.01$). Among these 9 patients there were 4 males and 5 females with a median age of 80 years (range 65–93).

5.3. Evaluation of risk factors for complications and mortality in PPU patients

The results of univariate analysis for the risk factors for complications and mortality (prospective multicentre studies, i.e. Copernicus and MEDWIS, publication III) are given in **Table (Table 4 in publication III,** Langenbeck's Arch Surg, 2000; 385: page 346) and for mortality (a retrospective Estonian study, publication II) are given in **Table (Table 5 in publication II,** Hepato-gastroenterol, 2000; 47: page 1767). Three variables, age ≥ 60 years ($P<0.001$), delay on admission ≥12 hours ($p<0.01$) and female sex ($p<0.05$) were the significant predictors for complications and mortality in the international study; five variables, age ≥65 years, female sex, treatment delay ≥12 hours, concomitant diseases and nondefinitive operation increased mortality risk on the basis of univariate analysis in the Estonian study. On the inclusion of these significant variables in multivariate analysis with a stepwise logistic regression, only age ≥60 years (OR 4.5, p=0.001) and delay on admission ≥12 hours (OR 2.5, p=0.012) in the international study, and only patients' age ≥65 years (OR 13.9, p=0.01) and

concomitant diseases (OR 10.7, p=0.01) in the Estonian study remained the significant (independent) predictors for complications and mortality in PPU patients.

5.4. Characteristics of PPU patients in a prospective study, possible reasons for high PPU incidence in Estonian patients (publication VII)

Altogether 129 PPU patients with a mean age of 48.6 years (96 males with a mean age of 42.8 and 33 females with a mean age of 65.6 years) were hospitalised at Tartu University Clinic in 1997–99. There were 111 DU and 16 GU perforations. Of the patients 30% had a history of confirmed ulcer disease before perforation (the diagnosis was confirmed endoscopically in 12%, on X-ray in 4%, with both methods in 12% and on a previous operation for perforation in 2% of the patients). The time interval from the primary diagnosis of ulcer disease till perforation varied from 2 months to 45 years (median 8 years). At the same time, 78% of all PPU patients and 44% of the patients with confirmed ulcer disease had not used any antiulcerogenic drugs during the previous year (**Table 2**), only 1 patient (3%) had used omeprazole and none of the 129 patients had received *H.pylori* eradication treatment. Of the patients 11% used NSAIDs daily (**Table 2**), 77% were smokers and 63% complained of elevated stress in everyday life (patients` subjective estimation). Of the patients 24% were pensioners, 41% had employment and 20% were unemployed or had only odd-jobs.

Table 2. Characteristics of 129 PPU patients hospitalised to Tartu University Hospital in 1997–1999.

Characteristic	%	Characteristic	%
1. Previous history		4. NSAIDs:	
– confirmed ulcer	30%	– daily	11%
– previous dyspeptic complaints	53%	– sometimes	25%
– no complaints	17%		
2. Antiulcer drugs usage (all patients):		5. Smoking	77%
– H2 blockers	21%	6. Increased stress in history	63%
– PPI	1%		
– *H. pylori* eradication	0%		
– without any treatment	78%		
3. Antiulcer drugs before perforation		7. Social status:	
(patients with confirmed ulcer):		– employed	41%
– PPI	3%	– unemployed/odd-job	20%
– H2 blocker, maintenance	5%	– pensioner	24%
– H2 blocker, intermittent	24%	– disabled person	10%
– H2 blocker, when needed	24%	– student/on maintenance	5%
– without any treatment	44%		

5.5. *H. pylori* in PPU patients

5.5.1. *H. pylori* infection rates in PPU patients

Presence of *H. pylori* in at least one gastric mucosal specimen out of four studied prior to eradication therapy was confirmed in 97% (93 of 96) of patients histologically. The bacteria were detected in the antral mucosa in 96% (51 of 53) of the patients using the PCR technique, while *H. pylori* was detected histologically in the antral mucosa of 52 out of 53 of these PPU patients. Thus in one patient with no detected *vacA* or *cagA* genotype, *H. pylori* was still proved to be present histologically.

5.5.2. Evaluation of different *H. pylori* strains in the Estonian and Russian patients (publication IV)

In one Estonian male with a *cagA* positive sample, two *vacA* gene subtypes (s1a/m1 and s1a/m2) were found simultaneously and the patient was excluded from further analysis. The distribution of the remaining 50 *H. pylori* positive samples according to the markers of *cagA* and *vacA* gene subtypes is presented in **Table (Table 2 in publication IV,** FEMS Microbiol Letters, 2001; 195: page 31). Among the *H. pylori* positive samples, 41 (82%) were *cagA* positive. The most frequent was the s1a/m1 *vacA* subtype (31 cases), while the s1b subtype was not found in PPU patients from South Estonia.

Both s1 subtypes (s1a/m1 and s1a/m2) prevailed (98%) in *cagA* positive samples. Comparison of the distribution of the pattern of the three *vacA* subtypes in patients with *cagA* positive and negative strains by using Chi-square test yielded a statistical difference at the level $p<0.001$ (**Table 2 in publication IV,** FEMS Microbiol Letters, 2001; 195: page 31).

No differences were observed in the distribution of *cagA* positive or negative markers between the Estonian and Russian patients with PPU, as the *cagA* gene was revealed in 81% (26 of 32) and 83% (15 of 18) of cases, respectively (**Figure 1 in publication IV,** FEMS Microbiol Letters, 2001; 195: page 31). In contrast, the distribution of *vacA* subtypes was different in the gastric samples of the Estonian and Russian patients studied. The s1a/m1 subtype was found in 75% (24 of 32) of the Estonians but in only 44% (8 of 18) of the Russians. At the same time, the s1a/m2 subtype was more frequent in the Russians (44%, 8 cases) than in the Estonians (13%, 4 cases). The s2/m2 subtype was detected in 4 (13%) Estonians and 2 (11%) Russians. Comparison of the distribution of the three *vacA* subtypes in the gastric mucosa samples of PPU patients of different nationalities by Chi-square test yielded a statistical difference ($p=0.037$).

No significant differences were revealed in the distribution of age, sex, smoking habits or ulcer location among the Estonian and Russian PPU patients studied.

5.5.3. Results of *H. pylori* eradication in PPU patients (publications No 5 and No 8)

The results of postoperative *H. pylori* eradication in 49 PPU patients (6 regimens) are presented in **Table 3.** In different regimens *H. pylori* was eradicated (histological estimation) in 0–37.5% of the patients, while in control group of PPU patients (no eradication attempted, i.e. only H-2 blocker or PPI was used) eradication was not observed in any patient.

Table 3. The results of postoperative *H. pylori* eradication after 2 months (histologically) in 49 PPU patients.

Regimen	No. of patients	Mean age (years)	Males/ females	Duodenal/ gastric ulcer	Eradication No. of pts. (%)
ACO* p/o 7 days	11	40,0	11/0	9/2	2 (18,2 %)
AMO* p/o 7 days	8	31,9	8/0	8/0	3 (37,5%)
AM** p/o 7 days	16	46,2	15/1	16/0	4 (25,0%)
AC** p/o 7 days	6	37,7	5/1	6/0	1 (16,7%)
C *** i/v 5 days	6	54,0	5/1	5/1	0 (0%)
C *** p/o 7 days	2	54,0	1/1	1/1	0 (0%)
No eradication***	10	46,1	8/2	6/4	0 (0%)

A – Amoxicillin — rat. 1g b.i.d
C – Clarithromycin (Klacid) 0.5 g b.i.d
M – Metronidazole 0.5 g q.i.d.
O – Omeprazole (Losec) 20 mg b.i.d
* – used only in nonvagotomised patients
**–AM and AC (i.e. without PPI) regimens used only in vagotomised patients
***– both vagotomised and nonvagotomised patients

5.6. *H. pylori* in patients with different gastric diseases: perforated peptic ulcer, peptic ulcer disease and chronic gastritis (publication VI)

Among the 156 patients infected with *H. pylori* strains, no s1b strains were found. Multiple *H. pylori* strains were detected in 5 (3.2%) of the 156 patients studied. Among the 151 remaining investigated samples the *cagA* gene was detected in 132 samples of 151 (87%). The relationship between *cagA* status,

vacA subtypes and patient's disease is shown in **Table (Table 2 in publication VI,** J Clin Microbiol, 2002; 40: page 299). In CG and PUD patients all *cagA*-negative isolates were associated with the s2/m2 genotype. In contrast, in the PPU group, 4 *cagA*-negative isolates exhibited the *vacA* genotype s1a and, conversely, one *cagA*-positive isolate exhibited the *vacA* genotype s2. However, statistically the PPU group did not differ from the CG and PUD groups ($p>0.05$), as a similar correlation was found between *cagA*-positivity and the *vacA* s1a type.

6. DISCUSSION

6.1. Epidemiology of PPU in Tartu county in 1981–2000

A sharp, 2.7-fold increase in PPU incidence was noted in Tartu in 1991–2000 compared with 1981–1990. The PPU incidence of 20.0 for the period 1991–2000 is at least 2–3 times as high as that reported from the Western countries, 2.3–10 (Aeberhard *et al.,* 1990; Hermansson *et al.,* 1997; Makela *et al.,* 1992). There were no considerable changes in the Estonian patients' mean age and sex distribution throughout the 20 years. However, the mean age of the studied patients was considerably lower (46.7 years vs 53–63 years) and the proportion of female patients (22.3% versus about 40–60%) lower compared with the figures given in most Western studies (Svanes *et al.,* 1993; Hermansson *et al.,* 1997; Makela *et al.,* 1992; Gunshefski *et al.,* 1990).

We consider that factors as social stress, low anti-ulcer drug usage, but possibly also specific smoking habits and high *H. pylori* infection rates in Estonian community could have a certain impact on such sharp increase in PPU incidence.

The role of stress factors has been associated with the rise of PPU incidence (Lam *et al.,* 1995). An increased frequency of ulcer perforations has been associated with acute society stress as occurring during air-raids in the UK in World War II (Jamieson, 1955; Stewart, 1942) and among prisoners-of-war captured during that time (Cleave, 1962). Lam *et al.* (1995) found a relationship between the increase in PPU and society stress when Hong Kong underwent significant socio-economic and political changes in 1962–85. In 1991 independence was re-established in Estonia, which led to profound socio-economic changes in the whole society: a drastic decline in economy and a fast increase in unemployment. Unemployment rate increased rapidly from 0.6% in 1990 to 7.6% in 1994 and reached 10.0% in 1996 (Statistical Yearbook of Estonia 1999). It is worth noting that the indirect parameters of social stress, e.g. suicide rate (Schneider, 1973), showed a similar trend — after a decrease in 1987–1990 (incidence 24.5–27.1) suicide rate has risen rapidly in Estonia since 1991 to attain the highest figures of the last century (incidence 41.0 in 1994) (Wasserman *et al.,* 1994).

The hypothesis of the role of social stress is also strongly supported by the occurrence of a positive correlation between PPU incidence and suicide rate in Estonia for 1981–2000. In both samples male patients dominated over females (male to female ratios for PPU patients were 4.1 and 3.3, and for suicide victims 3.5 and 4.5 for 1981–90 and 1991–2000, respectively).

Another point for discussion is use of anti-peptic-ulcer drugs. H_2 blockers and proton pump inhibitors were introduced later in our community than in most developed European countries. Use of modern anti-ulcer drugs in Estonia per capita was several times lower than in Stockholm county, Sweden, whereas

the number of operations for ulcer disease per capita in Estonia was almost 20 times higher in 1993–1995 (Kiivet *et al.,* 1998). There is evidence that smoking plays an important role in ulcer perforation, especially in younger patients (Svanes *et al.,* 1997). Although corresponding data were not available for all patients of this study, our prospective study has shown that 77% of the PPU patients from the same area were daily smokers in 1997–1999 (Sillakivi *et al.,* 2000). At the same time, the proportion of daily smokers in general adult population was only 29–36% in the period 1990–2000, being 42–52% for males and 15–23% for females (Kasmel *et al.,* 2001).

The role of the important aetiological factor of peptic ulcer, *H. pylori*, has remained controversial in PPU (Sebastian *et al.,* 1995; Reinbach *et al.,* 1993). There were no data for *H. pylori* infection in the PPU patients studied, but its prevalence in Estonia is higher than in the Western countries, affecting 78–87% of population in Southern Estonia (Maaroos, 1995; Vorobjova *et al.,* 1994). In the same geographical area the prevalence of *H. pylori* infection was found to be 93% in non-operated DU patients (Peetsalu *et al.,* 1991) and 97% in GU patients (Maaroos *et al.,* 1991). In a prospective study genotyping *H. pylori* strains, the bacteria were detected in 98% of the PPU patients from the same area studied in 1997–1999 (Sillakivi *et al.,* 2001).

Mortality of PPU patients in our study (mean 5.9% for 20 years) was considerably lower than in most recent studies (Hermansson *et al.,* 1997; Gunshefski *et al.,* 1990; Hamby *et al.,* 1993). Also, mortality rate did not change during the study period, differently from increasing trends established in Western studies (Svanes *et al.,* 1993; Gunshefski *et al.,* 1990). The lower mortality can be related mainly to the different (i.e. younger) structure of the studied PPU patient sample which did not reveal any increase in age.

In conclusion, a significant, 2.7-fold increase in PPU incidence occurred in Tartu county since 1991, while the annual incidences of PPU were statistically correlated with the suicide rates for the period 1981–2000. At the same time, the mean age and sex distribution of PPU patients as well as outcome did not display any change.

6.2. Comparison of the East European and German PPU patients: epidemiology, treatment strategies and outcome

Within two multicentre prospective studies, conducted at 12 centres in Eastern Europe and 11 centres in Germany and Austria, data were collected according to standardised forms, which allowed to compare the research results. Of all the studied patients with acute abdominal pain, 4.2% in Eastern Europe and 1.6% in Germany had the final diagnosis of PPU, which is comparable to the figures of several previously reported series (Ikonen *et al.,* 1983, De Dombal, 1988, Ohmann *et al.,* 1992).

The main distinctive characteristics of the PPU patients in the two studies were sex and ulcer localisation. The proportion of females among the German patients was 35.1% versus 17.1% among the East European patients, and the proportion of gastric ulcer perforations was 54.1% versus 23.5%. In the German study the proportion of females is closer to the figures reported from the Western countries in recent decades, 40–60% (Aeberhard *et al.,* 1990; Svanes *et al.,* 1993; Mäkela *et al.,* 1992; Hermansson *et al.,* 1997), while the respective proportion for Eastern Europe is clearly divergent. The higher proportion of gastric ulcers in the German study is in agreement with data indicating its increasing trend in Western patients (Aeberhard *et al.,* 1990; Svanes *et al.,* 1993).

Median age did not reveal any significant difference, being 43 years for the Copernicus patients and 49 years for the MEDWIS patients. Both figures are lower than the reported median age of 62–66 years (Svanes *et al.,* 1993; Hermansson *et al.,* 1997). However, the higher median age of the German female patients compared with that of their East European counterparts (73 years versus 53 years) can be explained by the above described trends in the Western countries.

The other variables, i.e. delay on admission, shock on admission and previously proved ulcer disease, did not reveal differences between the East European and the German patients. There were relatively few users of ulcerogenic drugs in either study (8.1% and 18.8%) compared with the reported proportion of 40–70% (Bulut *et al.,* 1996; Bliss *et al.,* 1991; Lanas *et al.,* 1997; Svanes *et al.,* 1996).

The distribution of different operations in both studies was quite different, definitive operations accounting for 16.1% in the German study and 41.1% in the East European study. At different East European hospitals the proportion of definitive surgery varied in the range 0–91%, while at five centres only non-definitive operations were used. At the same time, in the German study, among definitive operations only 5 gastric resections were performed, whereas in the Eastern Europe, besides 12 gastric resections, also 55 operations combined with vagotomy were used. The high proportion of definitive operations is a characteristic feature of Eastern Europe, while according to the data of recent reports from the Western countries the number of definitive procedures is diminishing rapidly (Hermansson *et al.,* 1997; Roher *et al.,* 1996; Al Assi *et al.,* 1994; Kozol, 1995, Blomgren, 1997). Overall complication rates, 18.3% in the Copernicus study and 27.0% in the MEDWIS study, did not reveal significant differences, however, the proportion of patients with general complications was 12.4% and 35.1% ($P<0.01$), respectively. Like the other variables, overall complication rate in German patients is closer to 30%–57% reported in most Western studies (Evans *et al.,* 1997; Gunshefski *et al.,* 1990; Wakayama *et al.,* 1994), while the figures for Eastern Europe are comparable to that reported by Boey, 14% (Boey *et al.,* 1982). Higher figures for general complications in German patients can be attributed to the older median age of the patient sample

of that study. The higher proportion of females with higher median age can play some role in the development of general complications, as a trend of increase in general complications with the ageing of patients has been brought out in the Western countries (Svanes *et al.,* 1993).

Differences in mortality, 13.5% for the MEDWIS patients and 2.4% for the Copernicus patients, can also be explained by the different age structure of the two patient samples. All patients who died were ≥65 years old. The mortality rate of 13.5% in the MEDWIS study was comparable to 16–31% reported from the Western countries in the 1990s (Bulut *et al.,* 1996; Hermansson *et al.,* 1997; Evans *et al.,* 1997), while the rate of 2.4% for the East European patients was significantly lower.

6.3. Evaluation of the risk factors for complications and mortality in PPU patients

Optimal management of patients with PPU is still a matter of discussion. Decrease in mortality rates, from 20–30% in the 1930s–1940s (18) to about 5–10% in the 1970s (Svanes *et al.,* 1993; Boey *et al.,* 1982; Mattingly *et al.,* 1980), was not observed in the 1980s–1990s (Svanes *et al.,* 1993; Horowitz *et al.,* 1989). Moreover, mortality figures as high as 17–31% can be found in the literature (Hermansson *et al.,* 1997; Hamby *et al.,* 1993; Bulut *et al.,* 1996; Irvin *et al.,* 1989). Svanes et al found that the increasing mean age of patients may conceal better results in the management of PPU patients, and hence mortality figures do not increase when adjusting to age groups (Svanes *et al.,* 1989).

There has been much discussion about the choice of surgical procedure in the case of emergency operation. In our study 245 nondefinitive operations with a mortality of 7.3% and 141 definitive operations with a mortality of 1.4% were performed. However, comparison of the patients of the two groups (**Table 4 in publication II,** Hepato-gastroenterol, 2000; 47: page 1767). revealed that the higher mortality rates for nondefinitive operations resulted from the effect of the surgeon's decision on the choice of operation. Patients with probable risk factors served as more eligible candidates for nondefinitive procedure, since it was thought to be safer. Our results of multifactorial analysis revealed that operative method had no independent effect on mortality. Numerous data give evidence of better long-term results after definitive operation: recurrence rate is lower and the patient's self-estimation of his/her condition is higher (Jordan *et al.,* 1995; Robles *et al.,* 1995; Peetsalu *et al.,* 1994). Several studies have confirmed that there is no reason to fear for higher mortality in an emergency situation when definitive procedures are applied (Gunshefski *et al.,* 1990; Hamby *et al.,* 1993; Welch *et al.,* 1986; McIntosh *et al.,* 1996); in this respect nondefinitive operations appear to have no advantages even in high risk patients

(Schein *et al.,* 1990; Bennett *et al.,* 1985; DiQuinzio *et al.,* 1992). Our results demonstrate agreement with the above investigations.

Different studies have described about ten risk factors predicting outcome and morbidity in PPU patients, the most frequent being old age, concomitant diseases, treatment delay and shock on admission (Boey *et al.,* 1982; Evans *et al.,* 1997; Mattingly *et al.,* 1980; Irvin, 1989; McIntosh *et al.,* 1996; Wakayama *et al.,* 1994), but also gastric ulcer location (Hamby *et al.,* 1993; Horowitz *et al.,* 1989; Svanes *et al.,* 1989), hospitalisation at the time of perforation (Hamby *et al.,* 1993), large diameter of perforation (Paunescu *et al.,* 1995), use of steroids (Hamby *et al.,* 1993), leukocyte rate lower than 9.500/mm^3 (Wakayama *et al.,* 1994) and reperforation (Evans *et al.,* 1997).

Univariate analysis of our data revealed that high age (≥65 years), concomitant diseases, treatment delay ≥12 hours, female sex and nondefinitive operation are the risk factors for mortality, whereas location of ulcer, shock on admission and previous ulcer history had no influence on mortality in the Estonian study. However, only high age and concomitant diseases remained significant in multivariate analysis. Apart from nondefinitive surgery discussed above, neither treatment delay nor female sex had an independent effect on mortality in multifactorial analysis.

In univariate analysis of the international study, age ≥60 years, delayed treatment ≥12 hours and female sex were significant in the development of complications, while in multivariate analysis, only high age and delayed treatment remained the independent predictors for complications. Complications were related neither to ulcer location nor to the operation method applied.

Wakayama et al. concluded that old age alone contributed to lethal outcome (Wakayama *et al.,* 1994), while others found that old age alone (without concurrent factors) did not predict outcome (Boey *et al.,* 1982, Evans *et al.,* 1997). Our results are in accordance with the studies where old age *per se* was found to predict outcome.

In conclusion, patients' high age and presence of concomitant diseases predicted outcome after surgical procedure performed for PPU. The complications were related to patients high age and treatment delay. The result did not depend on the fact whether definitive or nondefinitive operations were applied.

6.4. Characteristics of PPU patients in a prospective study: possible reasons for high PPU incidence in the patients

As in our retrospective study (Sillakivi *et al.,* 2002) the patients of this study were significantly younger, mean age 48 years vs 53–63 years in Western studies, and the proportion of women was smaller, 26% vs 40–69% in Western studies, than in most studies conducted in developed countries (Svanes *et al.,* 1993; Aeberhard *et al.,* 1990; Hermansson *et al.,* 1997; Hamby *et al.,* 1993).

However, the age and sex composition of the Estonian PPU patients were similar to that of the patients studied in 1997 in East European countries (Copernicus Programme: a study of acute abdominal pain performed in East Europe) (Sillakivi *et al.,* 2000).

The results of this study point to several factors the co-effect of which has obviously resulted in the sharp increase in PPU incidence during the last decade in comparison with developed countries:

1. The role of social stress: the sharp rise in the curve of PPU incidence since 1991 is similar to the rise in the curve of suicide incidence (Wasserman et al, 1994). An analogous significant rise in PPU incidence was also noted in Hong Kong in the period after the establishment of independence, when a significant increase in stress was recorded against the background of political-economic changes (Lam *et al.,* 1995).
2. Social factors: the number of representatives of the so-called lower social class among the patients was large (only 41% had steady job). Duodenal ulcer, in particular, has been associated with lower social classes (Elashoff *et al.,* 1980).
3. A large proportion of smokers among the patients (77%): the important role of smoking in development of PPU has also been pointed out in other studies (Svanes, 2000).
4. Inadequacy of previous diagnostics: in a very large proportion of the PPU patients (70%) ulcer disease had remained undiagnosed, although upper abdominal complaints, characteristic of ulcer disease, occurred in 53% of the patients. Probable reasons for this are also low disease awareness and poorer access to medical aid for some social groups.
5. Non-use of modern conservative ulcer treatment prior to the development of perforation: 44% of the patients with diagnosed ulcer disease had not received ulcer treatment during a year before perforation. Earlier studies have shown that long-term maintenance treatment with H_2 blockers reduces significantly the risk of perforation in comparison with non-treated ulcer patients (Penston, 1990). No patient had undergone *H. pylori* eradication treatment. Comparison of the results of this study with those of the study conducted in Estonia in 1995 (Labotkin, 1996) reveals no significant change. Also, a questionnaire carried out five years ago demonstrated that although 34–84% of the physicians considered maintenance treatment and *H. pylori* treatment appropriate, such treatment was mostly administered for only 0–20% of the patients. However, it should be noted that the results of the earlier study were obtained with the use of a questionnaire for doctors, while the results of this study were based on an interview concerning actually received treatment.

The results of our study show that the frequency of *H. pylori* in PPU patients is very high (97% vs 50–83% in developed countries) (Reinbach *et al.,* 1993; Sebastian *et al.,* 1995). However, it is not clear if the observed changes in PPU

incidence are also associated with the high *H. pylori* infection rate of the Estonian patients. The role of *H. pylori,* whose eradication has become a major argument in the advance of nondefinitive operative treatment tactics in ulcer disease, is not ultimately clear in the case of perforations, unlike it is, e.g. in the case of ulcer hemorrhage (Reinbach *et al.,* 1993; Sebastian *et al.,* 1995). Furthermore, Arakawa and coauthors consider it appropriate to distinguish, besides *H. pylori* negative ulcer disease, also separate *H. pylori*-causing ulcer and *H. pylori*-non-causing ulcer in the case of a positive bacterial finding — indicating the possibility that namely in populations with high *H. pylori* infection rate a large proportion of ulcer patients infected with the bacterium are characterised by the circumstance that application or non-application of eradication does not actually influence disease remission or recurrence (Arakawa *et al.,* 2000).

In conclusion, it can be said that the incidence of PPU in Estonia in the last decade was steadily high. Among different risk factors for the Estonian patients, one can bring out high *H. pylori* incidence (97%), stress, social problems, smoking (77% of PPU patients). Ulcer disease was diagnosed in most patients (70%) as late as on development of perforation. Nor was adequate modern conservative treatment available even for patients with previously diagnosed ulcer disease, while *H. pylori* eradication was not altogether applied in disease management.

6.5. *H. pylori* in PPU patients

6.5.1. Evaluation of different *H. pylori* strains in the Estonian and Russian PPU patients

We investigated the distribution of *H. pylori* genotypes in Southern Estonia among patients with complicated, histologically proven *H. pylori* associated peptic ulcer disease. Using PCR method the genotypes of *H. pylori* were estimated according to the presence of the *cagA* gene and *vacA* gene subtypes in specimens of the gastric antral mucosa.

That fact that we did not find any gastric mucosa sample containing the s1b subtype of the *vacA* gene among the 50 PPU patients investigated by us confirms the existence of geographical differences between the *H. pylori* genotypes. And, although the s1b subtype was not found in the *H. pylori* of Asian patients (Atherton *et al.,* 1996), its presence has been established in several studies conducted in Portugal, the Netherlands, and the USA (Atherton *et al.,* 1997; Campbell *et al.,* 1997; Pan *et al.,* 1999). However, as Estonia is situated in Eastern Europe, geographic differences between the *H. pylori* genotypes cannot be linked with different continents.

Furthermore, in our study the distribution of the s1a/m1, s1a/m2 and s2/m2 subtypes of *vacA* genes was statistically different in the Estonian and Russian patients ($p<0.05$), both groups living in South Estonia. This confirms data about the ethnic tropism of *H. pylori*, suggested by Campbell *et al.* (Campbell *et al.*, 1997). Yet these authors associated the differences between the *H. pylori* strains, colonising Polynesians and Europeans in New Zealand, with race-specific specialisation of *H. pylori* separate strains. However, this cannot be the reason for *H. pylori* related ethnic differences between Estonians and Russians, as both nationalities belong to the same race. We suppose that different predominant strains may be circulating among different ethnic groups in a particular area.

The Estonian and Russian PPU patients studied by us showed a similarity in the distribution of age, sex and smoking habits as well as ulcer localisation. Evidently, these factors are not closely associated with the ethnic tropism of *H. pylori*. Regrettably, we could not follow the socio-economic conditions in the case of our patients. In previous epidemiological studies these conditions were strongly associated with transmission of *H. pylori* infection. In Germany, *H. pylori* infection was established in 6.1% of native German children versus 44.8% of Turkish children (Bode *et al.*, 1998). However, the different incidence of *H. pylori* infection in populations living in the same country but having a different economic background does not explain the spread of genetically distinct *H. pylori* strains among these populations. Whether the other determinants, e.g. region/country of birth and childhood, or microbial adherence and host receptors, determine a particular distribution of different genotypes of *H. pylori* should be established in further studies with a larger number of subjects.

The virulence markers differentiating *H. pylori* strains in patients with complicated and uncomplicated peptic ulcer have not been assessed up to now. In our study the *cagA* gene of the *H. pylori* genome was found in 82% of PPU patients. The *cagA* status was similar in samples from the Estonian and Russian patients with the same underlying disease, while the *vacA* s1 subtype prevailed in both nationalities (98%). High prevalence of the $cagA^+$ genotype and the *vacA* s1 subtype has been associated with increased virulence of *H. pylori* strains (Atherton *et al.*, 1995, Atherton *et al.*, 1997). However, recently we found a predominance of the *vacA* s1 gene in another sample of South-Estonian patients suffering either from chronic active gastritis or peptic ulcer disease (Lõivukene *et al.*, 2000). It is likely that the presence of *cagA* and *vacA* s1 genes of *H. pylori* strains is not exclusively specific for complicated peptic ulcer disease but may merely reflect the circulation of predominant strains in a particular geographic region of Estonia, causing severe gastric diseases.

In conclusion, we found geographic and ethnic differences in *H. pylori* among patients with PPU from Estonia. This finding points to the need of further investigations aimed at the future development of novel therapeutic targets and vaccines specific for different ethnic groups.

6.5.2. Results of *H. pylori* eradication in PPU patients

In our study, *H. pylori* eradication was observed in a considerably smaller proportion of the PPU patients than expected and this even with the use of two classical triple treatment schemes well known worldwide: scheme I (amoxicillin, chlarithromycin and omeprazole), belonging also to the treatment schemes recommended by the Estonian Gastroenterologists' Society (Labotkin *et al.*, 1999), yielded a positive eradication result in only 18.2% of the cases; scheme II (metronidazole, amoxicillin and omeprazole), which yielded a positive result in 37.5% of the cases (Table 1). The other modified treatment schemes employed by us led to an unsatisfactory result as well — eradication in 0–25% of the cases.

Although chlarithromycin monotherapy was unsuccessful, in our opinion, the idea of immediate postoperative *H. pylori* eradication in complicated peptic ulcer patients seems to deserve further research, especially when combining several antibiotics and acid suppressing compounds.

Why eradication in this study occurred in such a small proportion of the patients remains unclear. As the possible reasons, the following circumstances can be considered:

1. Weekly peroral treatment courses applied by us for PPU patients in the postoperative period, which have yielded positive results in uncomplicated ulcer patients in Estonia as well, may not be suitable in PPU, or else the patients of this group require a longer treatment course. Yet the combination amoxicillin + chlarithromycin + omeprazole, used worldwide, belongs to the list recommended by the Estonian Gastroenterologists' Society and has yielded 91% eradication in uncomplicated PPU patients (Kolk *et al.*, 1998).
2. Initiation of treatment on the 7^{th}–10^{th} postoperative day in the PPU patients is too early. However, this disagrees with the results of other investigators according to which *H. pylori* treatment already from the 3^{rd} postoperative day was successful in 83% of PPU patients (Ng *et al.*, 2000).
3. Problems related to compliance, which has been regarded among the most important reasons for *H. pylori* eradication failure (Freston, 2000). It is evident that an outpatient setting does not provide the possibility for direct control of the actual use of medicines. However, as the patients studied by us attended outpatient follow-up at 2–3 months after the treatment course and consented to undergo an inconvenient procedure, endoscopy, it shows cooperation on the part of the patients and their interest in *H. pylori* eradication result.

As the reasons for eradication failure, one should also consider the large proportion of smokers (77%) among the PPU patients and their low mean age. These factors have also been reported to increase eradication failure rates (Labenz *et al.*, 1995; Treiber *et al.*, 2002; Haruma *et al.*, 1999)

The circumstance that according to a PCR study, eradication was observed at 2 months in only 41% (15 of 37) of the PPU patients treated with the use of different management schemes, should also be noted (unpublished, MSc thesis by H. Andreson).

The results of our study demonstrate that establishment of an effective treatment scheme, suitable for PPU patients, requires further research. Clarification of the specificity of *H. pylori* strains in PPU might offer new solutions for treatment problems as well.

Summing up, all treatment schemes used for *H. pylori* eradication in this study, including the combination which is popular worldwide and is currently recommended by Estonian gastroenterologists, have not resulted in the expected effect in PPU patients. Considering the high PPU incidence and the 97% *H. pylori* infection rate of these patients, elaboration of treatment tactics, suitable in the conditions of Estonia, requires further investigations in order to avoid postoperative ulcer recurrence and consequent development of life-threatening complications.

6.6. *H. pylori* in patients with different gastric diseases: perforated peptic ulcer, uncomplicated peptic ulcer disease and chronic gastritis

A study by Vorobjova *et al.* (Vorobjova *et al.,* 1998) demonstrated a high prevalence of *cagA* in a sample population of Estonian adults (63%) and children (46%). We found that the distribution of the *cagA* gene among the large sample of patients with different clinical diagnosis (PPU, PUD, CG) was very high (87%). Our study indicates that the *cagA* gene of *H. pylori* and its defined protein are the important predictors of several gastric diseases, even in populations with a high prevalence of infection like Estonia.

According to a large number of studies (Atherton *et al.,* 1995; Atherton *et al.,* 1997; Cover *et al.,* 1994; Evans *et al.,* 1998; van Doorn *et al.,* 1998), the *vacA* s1a/m1 subtype is considered more cytotoxic and is therefore more strongly related to development of PUD than the s1a/m2 and s2/m2 *vacA* genotypes. The s2 strains are rarely associated with peptic ulcer disease and are more common in cases of chronic gastritis or non-ulcer dyspepsia (Evans *et al.,* 1998; Gusmão *et al.,* 2000; Rudi *et al.,* 1999; Strobel *et al.,* 1998; van Doorn *et al.,* 1998). In our study there was no difference in colonisation by *H. pylori* cytotoxic strains between the patients with uncomplicated PUD and with PPU.

In conclusion, we established a high prevalence of the *cagA* gene of *H. pylori* among patients with different gastric diseases. However, no difference existed in the distribution of the *cagA* and *vacA* genotypes between patients with PPU, uncomplicated PUD and chronic gastritis in Estonia. This fact refers to the possibility of the clonal spread of a virulent *H. pylori* strain in Estonia, which needs to be elucidated.

7. CONCLUSIONS

1. A pronounced (2.7-fold) increase in PPU incidence took place in Tartu county from the period 1981–90 to 1991–2000, while the annual incidences of PPU were significantly correlated with the annual suicide incidences for 1981–2000. No significant changes were observed in PPU patients' mean age, sex distribution and outcome during the two periods compared. The PPU incidence of 20.0 for the period 1991–2000 is at least 2–3 times as high as that reported from developed countries.
2. In our studies, Estonian PPU patients were younger, the proportion of female patients was lower, and the proportion of definitive operations was higher compared with the German study or with reports from developed countries. The age and sex distribution of the Estonian patients studied and the use of surgical methods in treatment and outcome are similar to those observed in the East European study.
3. The high PPU incidence in Tartu county (and in Estonia) in the recent decade is evidently related to increased (social) stress, cigarette smoking and the lack of modern effective antiulcer therapy for patients with ulcer disease. To reduce the incidence of this life-threatening complication, proper measures should be directed to the raising of the awareness of both the medical staff and the public.
4. Patients' high age (≥65 years) and the presence of concomitant diseases are the independent predictors of lethal outcome after surgical procedure performed for PPU. Age ≥ 60 years and delay on admission ≥ 12 hours are the significant predictors for complications in PPU patients. The mortality and complication rates are not influenced by the fact whether definitive or nondefinitive operation is applied.
5. *H. pylori* infection is present in 97% of the Estonian PPU patients. *H. pylori* infection is characterized by geographic and ethnic differences among the PPU patients: the distribution of the *vacA* gene subtypes is statistically different in the Estonian and Russian patients, and the s1b subtype of the *vacA* gene which is widespread in some other countries, is lacking in our patients. This finding points to the need for further investigations aimed at the development of novel therapeutic targets and vaccines specific for different ethnic groups.
6. A high (87%) prevalence of the *cagA* gene of *H. pylori* is characteristic of patients with different gastric diseases from Southern Estonia. The distribution of the virulence markers, particulary the *cagA* gene and *vacA* genotypes, is not different for patients with PPU, for those with uncomplicated PUD and for those with chronic gastritis in Estonia.
7. The present results of *H. pylori* eradication in the Estonian PPU patients have been unsatisfactory with the use of all studied regimens, indicating that PPU cannot be managed using ordinary *H. pylori* eradication schemes. Detailed classification of the reasons for management failure as well as development of the treatment tactics suitable for the Estonian conditions requires further research.

8. REFERENCES

Adamek RJ, Wegener M, Labenz J, Freitag M, Opferkuch W, Ruhl GH. Medium-term results of oral and intravenous omeprazole/amoxicillin Helicobacter pylori eradication therapy. Am J Gastroenterol 1994; 89 (1): 39–42.

Aeberhard P, Lichtenhahn P, Villiger P. Heutiger Stand der Therapie des perforierten Gastroduodenalulkus. Schweiz Med Wschr. 1990; 120: 467–475.

Al-Assi MT, Graham DY. Peptic ulcer disease, Helicobacter pylori, and the surgeon: Changing of the guard. Curr Opin Gen Surg 1994: 120–124.

Andersen LP, Dorland A, Karacan H, Colding H, Nilsson HO, Wadström T, Blom J. Possible clinical importance of the transformation of *Helicobacter pylori* into coccoid forms. Scand J Gastroenterol 2000; 35: 897–903.

Andersen, IB, Jørgensen T, Bonnevie O, Grønbaek MN, Sørensen TI. Tobacco and alcohol are risk factors of complicated peptic ulcers. A prospective cohort study. Ugeskrift for Laeger 2001: 163 (38, Sept. 17): 5194–5199.

Arakawa T, Higuchi K, Fujiwara Y, Tominaga K, Watanabe T, Shiba M, Uchida T, Kuroki T. Helicobacter pylori: criminal or innocent bystander? Gastroenterol 2000; 35 (Suppl 12): 42–46.

Atherton JC, Cao P, Peek RM, Tummuru MKR., Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. J Biol Chemistry 1995; 30, 1771–1777.

Atherton JC, Hawkey CJ, Spiller RC, Cockayne A, Balsitis M, Kirk GE. Detection of the intragastric sites at which Helicobacter pylori evades treatment with amoxycillin and cimetidine. Gut 1995; 36 (5): 670–674.

Atherton JC, Karita M, Gonzalez-Valencia G, Peek RM, Cover TL. Diversity in vacA mid-region sequence but not in signal sequence type among *Helicobacter pylori* strains from Japan, China, Thailand and Peru. Gut 1996; 39: A73–A74.

Atherton JC, Peek RM, Tham KT, Cover TL, Blaser MJ. Clinical and pathological importance of heterogeneity in VacA, the vacuolating cytotoxin gene of *Helicobacter pylori*. Gastroenterol 1997; 112: 92–99.

Axon ATR. Helicobacter pylori therapy: effect on peptic ulcer disease. J Gastroenterol Hepatol 1991; 6: 131–137.

Beales ILP. Claim for major advance in treatment of perforated peptic ulcer seems premature. BMJ 1998; 316: 860–861.

Beales ILP. Helicobacter pylori and peptic ulcer surgery. Br J Surg 1998; 85: 571.

Bennett KG, Cannon JP, Organ CH. Is duodenal ulcer perforation best treated with vagotomy and pyloroplasty? Am J Surg 1985; 150: 743–747.

Berstad K, Berstad A. *H. pylori* infection in peptic ulcer disease. Scand J Gastroenterol 1993; 28: 561–567.

Bliss DW, Stabile BE. The impact of ulcerogenic drugs on surgery for the treatment of peptic ulcer disease. Arch Surg 1991; 126: 609–612.

Blomgren LG. Perforated peptic ulcer: long-term results after simple closure in the elderly. W J Surg 1997; 21: 412–415.

Bode G, Rothenbacher D, Brenner H, Adler G. *Helicobacter pylori* and abdominal symptoms: a population-based study among preschool children in Southern Germany. Paediatrics 1998; 101: 634–637.

Bodner B, Harrington ME, Kim U. A multifactorial analysis of mortality and morbidity in perforated peptic ulcer disease. Surg Gynecol Obstet 1990; 171: 315–320.

Boey J, Wong J, Ong GB. A prospective study of operative risk factors in perforated duodenal ulcers. Ann Surg 1982; 195: 265–269.

Boey J, Choi SKY, Alagaratnam TT, Poon A. Risk Stratification in Perforated Duodenal Ulcers. A Prospective Validation of Predictive Factors. Ann Surg 1987; 205: 22–6.

Bulut O, Rasmussen C, Fischer A. Acute surgical treatment of complicated peptic ulcers with special reference to the elderly. World J Surg 1996; 20: 574–577.

Campbell S, Frazer A, Holliss B, Schmid J, O'Toole PW. Evidence for ethnic tropism of *Helicobacter pylori.* Infect Immun 1997; 65: 3708–3712.

Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A. *Cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc Natl Acad Sci 1996; 93: 14648–14653.

Chan WH, Wong WK, Khin LW, Soo KC. Adverse operative risk factors for perforated peptic ulcer. Annals of the Academy of Medicine, Singapore 2000; 29 (2): 164–167.

Chowdhary SK, Bhasin DK, Panigrahi D, Malik AK, Kataria RN, Behra A, Roy P, Singh K. Helicobacter pylori infection in patients with perforated duodenal ulcer. Tropical Gastroenterology 1998; 19: 19–21.

Cleave TL. Evidence from prisoner-of-war camps in the Far East, 1942–45. In: Peptic Ulcer: A new Approach based on Causation, Prevention and Arrest. Basic Human Evolution. Bristol: John Wright and Sons Ltd. 1962: 64–74.

Cocks JR. Perforated peptic ulcer — the changing scene. Dig Dis 1992; 10: 10–16.

Coggon D, Lambert P, Langman MJS. 20 years of hospital admissions for peptic ulcer in England and Wales. Lancet 1981;1:1302–1304.

Cover TL, Blaser MJ. Purification and characterization of the vacuolating toxin from *Helicobacter pylori.* J Biol Chem 1992; 267: 10570–10575.

Cover TL, Dooley CP, Blaser MJ. Characterization of human serologic response to proteins in *Helicobacter pylori* broth culture supernatants with vacuolizing cytotoxin activity. Infect Immun 1990; 58: 603–610.

Cover TL, Tummuru MKR, Cao P, Thompson SA, Blaser MJ. Divergence of genetic sequences for the vacuolating cytotoxin among *Helicobacter pylori* strains. J Biol Chem 1994; 269: 10566–10573.

Crisp E. Cases of perforation of the stomach. Lancet 1843; 1: 639.

Crofts TJ, Park KGM, Steele RJC, Chung SSC, Li AKC. A randomized trial for nonoperative treatment for perforated peptic ulcer. N Engl J Med 1989; 320: 970.

Creuveilhier J. Maladies de l`Estomac, De l`Anatomie Pathologique du Corps Humain. Vol.2. Paris:Bailliere, 1835.

Cullen DJE. Collins BJ, Christiansen KJ. When is Helicobacter pylori infection acquired? Cut 1993; 34: 1681–1682.

De Boer WA. Perforated Duodenal ulcer. N Engl J Med 1997; 337: 1013.

De Dombal FT. The OMGE acute abdominal pain survey. Progress Report 1986. Scand J Gastroenterol 1988; 23 (suppl 144): 35–42.

DiQuinzio C, Phang PT. Surgical management of perforated benign gastric ulcer in high-risk patients. Can J Surg 1992; 35: 94–97.

Donderici O, Temizhan A, Kucukbas T, Eskioglu E. Effect of Ramadan on peptic ulcer complications. Scand J Gastroenterol 1994; 29 (7): 603–606.

Donelli G, Matarrese P, Fiorentini C, Dainelli B, Taraborelli T, Di Campli E, Di Bartolomeo S, Cellini L. The effect of oxygen on the growth and cell morphology of *Helicobacter pylori*. FEMS Microbiol Letters 1998; 168: 9–15.

Donovan AJ, Berne TV, Donovan JA. Perforated duodenal ulcer. Arch Surg 1998; 133: 1166–1171.

Druart ML, Van Hee R, Etienne J, Cadiere GB, Gigot JF, Legrand M, Limbosch JM, Navez B, Tugilimana M, Van Vyve E, Vereecken L, Wibin E, Yvergneaux JP. Laporoscopic repair of perforated duodenal ulcer. A prospective multicenter clinical trial. Surg Endosc 1997; 11: 1017–1020.

Elashoff JD, Grossmann MT. Trends in hospital admissions and death rates for peptic ulcer in the United States from 1970 to 1978. J Clin Gastroenterol 1980; 78: 280–285.

European Helicobacter Pylori Study Group: Current European Concepts on the Management of *Helicobacter Pylori* Infection. The Maastricht Consensus Report 1996.

Evans DG, Queiroz DMM, Mendes EN, Evans DJ. *Helicobacter pylori cagA* status and s and m alleles of *vacA* in isolates from individuals with a variety of *H. pylori* — associated gastric diseases. J Clin Microbiol 1998; 36: 3435–3437.

Evans JP, Smith R. Predicting poor outcome in perforated peptic ulcer disease. Aust NZ J Surg 1997; 67: 792–795.

Freston JW. Management of peptic ulcers: Emerging issues. World J Surg 2000; 24: 250–255

Friess H, Malfertheiner P, Flock F, Baczako K, Stanescu A, Büchler M. Elimination of *H. pylori* by single shot antibiotic treatment in patients undergoing proximal gastric vagotomy. Eur J Gastroenterol & Hepatol 1992; 4: 719–725.

Glupczinski YS, Goutier C, van den Borre S, Butzler JP, Burette A. Surveillance of Helicobacter pylori resistance to antimicrobial agents in Belgium from 1989 to 1994. Gut 1995; 37 (Suppl1): A56.

Glupczynski Y. Culture of *Helicobacter pylori* from gastric biopsies and antimicrobial susceptibility testing. In: Megraud F, Lee A (ed.), *Helicobacter pylori*: techniques for clinical diagnosis. W. B. Saunders Company Ltd., London, United Kingtom 1996: 17–32.

Graham DY, Lew GM, Malaty HM, Evans DJ, Klein PD, Alpert LC, Genta RM. Factors influencing the eradication of Helicobacter pylori with triple therapy. Gastroenterol 1992; 93: 553.

Gunshefski L, Flancbaum L, Brolin RE, Frankel A. Changing patterns in perforated peptic ulcer disease. Am Surg 1990; 56: 270–274.

Gusmão VR, Mendes EN, Queiroz DMM, Rocha GA, Rocha AMC, Ashour AAR, Carvalho AST. *VacA* genotypes in *Helicobacter pylori* strains isolated from children with and without duodenal ulcer in Brazil. J Clin Microbiol 2000; 38: 2853–2857.

Hamby LS, Zweng TN, Strodel WE. Perforated gastric and duodenal ulcer: an analysis of prognostic factors. Am Surg 1993; 59: 319–324.

Haruma K, Kamada T, Kitadai Y, Chen X, Kido S, Hamada H, Mihara M, Tanaka S, Yoshihara M, Sanuki E, Sumii K, Kajiyama G. Smoking affects the eradication rate of Helicobacter pylori with omeprazole, amoxicillin and clarithromycin. Gut 1999; 45 (Suppl 111): A120.

Hermansson M, Stael von Holstein C, Zilling T. Peptic ulcer perforation before and after the introduction of H_2-receptor blockers and proton pump inhibitors. Scand J Gastroenterol 1997; 32: 523–529.

Hölscher AH, Gutschow C, Schäfer H, Bollschweiler E. Conventional surgery in peptic ulcer perforation: indications and procedure. Kongressband / Deutsche Gesellschaft für Chirurgie. Kongress Volume 118, 2001: 285–288.

Horowitz J, Kukora JS, Ritchie WP. All perforated ulcers are not alike. Ann Surg 1989; 209: 693–696.

Ikonen JK, Rokkanen PU, Grönroos P, et al. Presentation and diagnosis of acute abdominal pain in Finland: a computer aided study. Ann Chir Gynaecol 1983; 72: 332–336.

Iovene MR, Romano M, Pilloni AP, Giordano B, Montella F, Caliendo S, et al. Prevalence of antimicrobial resistance in eighty clinical isolates of H.pylori. Chemother 1999; 45: 8–14.

Irvin TT. Mortality and perforated peptic ulcer: A case for risk stratification in elderly patients. Br J Surg 1989; 76: 215–218.

Ito Y, Azuma T, Ito S, Miyaji H, Hirai M, Yamazaki Y, Kohli Y, Kuriyama M. Analysis and typing of the vacA gene from cagA-positive strains of *Helicobacter pylori* isolated in Japan. J Clin Microbiol 1997; 35: 1710–1714.

Jamieson RA. Acute perforated peptic ulcer. Frequency and incidence in the West of Scotland. BMJ 1955; 2: 222–227.

Jordan PH, Thornby J. Perforated pyloroduodenal ulcers. Long-term results with omental patch closure and parietal cell vagotomy. Ann Surg 1995; 221: 479–488.

Kasmel A, Lipand A, Markina A, Kasmel K.Health behaviour among Estonian adult population, spring 2000. OÜ Dada AD, Tallinn, Estonia, 2001. Table 30.B.

Khorsovani C, Kohen M, Guiberteau B, Le Neel JC. Perforation of duodenopyloric ulcers. Prognostic factors and therapeutic choices. Retrospective study of 140 patients. Ann Chir 1994; 48: 345–349.

Kiivet R-A, Bergman U, Sjostedt S, Sjoqvist F. Ulcer surgery in Estonia, a consequence of drug delay? Lancet 1998; 351: 146.

Klein PD, Graham DY, Gaillour A, Opekun AR, Smith EO. Gastrointestinal Physiology Working Group. Water source as risk factor for *Helicobacter pylori* infection in Peruvian children. Lancet 1991; 337: 1503–1506.

Kolk H, Maaroos H-I, Labotkin K, Kull I, Lõivukene K, Mikelsaar M, Lindberg G, Ahokannas M. *Helicobacter pylori* reinfektsioon. Arstiteaduskonna aastakonverentsi teesid, Tartu 1998: 29

Kosunen TU, Hook J, Rautelin HI, Myllyla G. Age-dependent increase of Helicobacter pylori antibodies in blood donors. Scand J Gastroenterol 1989; 24: 110–114.

Kozol RA. Surgery for peptic ulcer in the Helicobacter pylori era. Arch Surg 1995; 130: 1040–1041.

Kurata JH, Haile BM. Epidemiology of peptic ulcer disease. Clin Gastroenterol 1984; 13:289–305.

Labenz J, Jorias I, Sollbohmer M, Borsch G, Stolte M, Blum A, Leverkus F, Bertrams J. Intragastric acidity as a predictor of the success of Helicobacter pylori eradication: a study in peptic ulcer patients with omeprazole and amoxicillin. Gut 1995; 37 (1): 39–43.

Labotkin K, Maaroos H-I, Salupere R. *Helicobacter pylori* infektsiooni ravi juhend. Eesti Arst 1999; 3: 280–282.

Labotkin K. Haavandtõve medikamentoosne ravi Eestis. Eesti Arst 1996; 5: 387–392.

Laine L, Hopkins RJ, Girardi LS. Has the impact of Helicobacter pylori therapy on the ulcer recurrence in the United States been overstated? A meta-analysis of rigorously designed trials. Am J Gastroenterol 1998; 93: 1409–1415.

Laine L. The long-term management of patients with bleeding–ulcers: *Helicobacter pylori* eradication instead of maintenance antisecretory therapy. Editorial. Gastrointest Endosc 1995; 41: 77–79.

Lam SK, Hui WM, Shiu LP, Ng MM. Society stress and peptic ulcer perforation. J Gastroenterol Hepatol 1995; 10: 570–576.

Lanas A, Serrano P, Bajador E, Esteva F, Benito R, Sainz R. Evidence of aspirin use in both upper and lower gastrointestinal perforation. Gastroenterol 1997; 112: 683–689.

Langman MJS.The epidemiology of chronic digestive disease. Edward Arnold, London 1979: 9–38.

Lanng C, Palnaes Hansen C, Christensen A, Thagaard CS, Lassen M, Klaerke A, Tonnesen H, Ostgaard SE. Perforated gastric ulcer. Br J Surg 1988; 75: 758–759.

Lau WY, Leung KL, Zhu XL, Lam YH, Chung SC, Li AK. Laparoscopic repair of perforated peptic ulcer. Br J Surg 1995; 82: 814–816.

Lindkvist P. Risk factors for infection with *Helicobacter pylori*. Doktoriteesid. Stockholm, 1999.

Logan RPH, Walker MM, Misiewicz JJ, Gummett PA, Karim QN, Baron JH. Changes in the intragastric distribution of *Helicobacter pylori* during treatment with omeprazol. Gut 1995; 36:12–16.

Lutsuver A. Comparative assessment of different methods of surgical intervention in perforated pyloroduodenal ulcers (in Russian). Cand. Sci (med.) thesis. Tartu State University, Tartu, 1978.

Lõivukene K, Kolk H, Maaroos H-I, Kasenõmm P, Aro H, Ustav M, Mikelsaar M. Metronidazole and clarithromycin susceptibility and the subtypes of *vacA* of *Helicobacter pylori* isolates in Estonia. Scand J Infect Dis 2000; 32: 59–62.

Lõivukene K, Maaroos H-I, Kolk H, Kull I, Labotkin K, Mikelsaar M. Prevalence of antibiotic resistance of *Helicobacter pylori* isolates in Estonia during 1995–2000 in comparison to the consumption of antibiotics used in treatment regimens. Clin Microbial Infect 2002;8:598–603.

Maaroos H-I, Kekki M, Sipponen P, Salupere V, Villako K. Grade of *Helicobacter pylori* colonisation, chronic gastritis and relative risks of contracting high gastric ulcers: a seven-year follow-up. Scand J Gastroenterol 1991; 26 (Suppl 186): 65–72.

Maaroos H-I: Helicobacter pylori infection in Estonian population: is it a health problem? Ann Med 1995; 27: 613–616.

Maeda S, Ogura K, Yoshida H, Funai F, Ikenoue T, Kato N, Shiratori Y, Omata M. Major virulence factors, VacA and CagA, are commonly positive in *Helicobacter pylori* isolates in Japan. Gut. 1998; 42: 338–343.

Maher W, Jyotheeswaran S, Potter G, Shaw A, Malkin M, Joseph D, Wagner D, Faiello P, Chey WY. An epidemiological study of peptic ulcer disease patients in greater Rochester. New York. Gastroenterol 1997; 113: A206.

Mäkelä J, Laitinen S, Kairaluoma MI. Complications of peptic ulcer disease before and after the introduction of H_2-receptor antagonists. Hepatogastroenterol 1992; 39: 144–148.

Malaty, H.M., D.G. Evans, D.J. Evans, and D.Y. Graham. *Helicobacter pylori* in Hispanics: comparison with blacks and whites of similar age and socioeconomic class. Gastroenterology 1992; 103: 813–16.

Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983; i: 1273–1275.

Matsukura N, Onda M, Tokunaga A, Kato S, Yoshiyuki T, Hasegawa H, Yamashita K, Tomtitchong P, Hayashi A. Role of Helicobacter pylori infection in perforation of peptic ulcer: an age- and gender-matched case-control study. J Clin Gastroenterol 1997; 25 (Suppl 1): S235–9.

Mattingly SS, Ram MD, Griffin WO Jr. Factors influencing morbidity and mortality in perforated duodenal ulcer. Am Surg 1980; 46: 61–66.

May JM. Report on the geography of peptic ulcers. Schweiz Z Path Bakt 1985; 21:169–209.

McColl KEL. Helicobacter pylori: Clinical aspects. J Infection 1997; 34: 7–13.

McIntosh JH, Berman K, Holliday FM, Byth K, Chapman R, Piper DW. Some factors associated with mortality in perforated peptic ulcer: A case control study. J Gastroenterol Hepatol 1996; 11: 82–87.

Megraud F. Epidemiology and mechanism of antibiotic resistance in *H. pylori.* Gastroenterol 1998; 115: 1278–1282.

Megraud F. Resistance of H.pylori to antibiotics. Aliment Pharmacol Ther 1997; 11: 43–53.

Mihmanli M, Isgor A, Kabukcuoglu F, Turkay B, Cikla B, Baykan A. The effect of H. pylori in perforation of duodenal ulcer. Hepato-Gastroenterol 1998; 45: 1610–1612.

Mikulicz J. Über Laparotomie bei Magen und Darmperforation. Samml Klin Vort. Leipzig 1885; 262: 2307.

Misiewicz, JJ, Tytgat GNJ, Goodwin CS, et al. The Sydney system: A new classification of gastritis. World Congress on Gastroenterology. Working Party Reports. Sydney, Blackwell Science Publishing 1990: 1–10.

Moayyedi P, Chalmers DM, Axon AT. Patient factors that predict failure of omeprazole, clarithromycin, and tinidazole to eradicate Helicobacter pylori. Gastroenterol 1997; 32 (1): 24–27.

Nakamura A, Park A, Nagata K, Sato EF, Kashiba M, Tamura T, and Inoue M. Oxidative cellular damage associated with transformation of *Helicobacter pylori* from a bacillary to a coccoid form. Free Radic Biol Med 2000; 28: 1611–1618.

Ng EKW, Lam YH, Sung JJY, Yung MY, To KF, Chan ACW, Lee DWH, Law BKB, Lau JYW, Ling TKW, Lau WY, Chung SCS. Eradication of *Helicobacter pylori* prevents recurrence of ulcer after simple closure of duodenal ulcer perforation. Ann Surg 2000; 231: 153–158.

Ng EK, Chung SC, Sung JJ, Lam YH, Lee DW, Lau JY, Ling TK, Lau WY, Li AK. High prevalence of Helicobacter pylori infection in duodenal ulcer perforations not caused by non-steroidal anti-inflammatory drugs. Br J Surg 1997; 84: 1029–1030.

Ohmann C, Kraemer M, Jäger S, *et al.* Akuter Bauchschmerz — standardisierte Befundung als Diagnose-unterstützung. Chirurg 1992; 63: 113–123.

Owen RJ. Helicobacter—species classification and identification. Br Med Bull 1998;54(1):17–30.

Paimela H, Tuompo PK, Peräkylä T, Saario I, Höckerstedt K, Kivilaakso E. Peptic ulcer surgery during the H_2-receptor antagonist era: a population-based epidemiological study of ulcer surgery in Helsinki from 1972–1987. Br J Surg 1991; 78: 28–31.

Pan ZJ, Berg DE, van der Hulst RW, Su WW, Raudonikiene A, Xiao SD, Dankert J, Tytgat GN, van der Ende. A. Prevalence of vacuolating cytotoxin production and distribution of distinct *vacA* alleles in *Helicobacter pylori* from China. J Infect Dis 1998; 178: 220–226.

Pan ZJ, van der Hulst RWM, Feller M, Xiao SD, Tytgat GNJ, Dankert J, van der Ende A. Equally high prevalence of infection with cagA-positive *Helicobacter pylori* in Chinese patients with peptic ulcer disease and those with chronic gastritis associated dyspepsia. J Clin Microbiol 1997; 35: 1344–1347.

Pan ZJ, van der Hulst WM, Tytgat GNJ, Dankert J, van der Ende A. Relation between vacA subtypes, cytotoxin activity, and disease in *Helicobacter pylori*-infected patients from the Netherlands. Am J Gastroenterol 1999; 94; 1517–1521.

Paunescu V, Spircu T. Risk factors in the outcome of a perforated gastric ulcer: a multifactorial analysis. Br J Surg 1995; 82: 51.

Peetsalu A, Maaroos H-I, Sipponen P, Peetsalu M. Long-term effect of vagotomy on gastric mucosa and *Helicobacter pylori* in duodenal ulcer patients. Scand J Gastroenterol 1991, 26 (suppl 186): 77–83.

Peetsalu A, Peetsalu M, Vardja T. Long-term results after surgical treatment of perforated duodenal ulcer. Research in medicine 1991. Proceedings of the meeting, Tartu, 10 Oct. 1991: 70.

Peetsalu A, Vardja T, Peetsalu M, Väli T. Long-term results of surgical treatment of bleeding duodenal ulcers. Br J Surg 1994; 81: 57.

Peetsalu M, Maaroos H-I, Peetsalu A. Completeness of vagotomy, Helicobacter pylori colonization and recurrent ulcer 9 and 14 years after operation in duodenal ulcer patients. Eur J Gastroenterol and Hepatol 1998; 10: 305–311.

Peitz U, Wolle K, Sulliga M, Sehring M, von Arnim U, Leodolter A, Kahl S, Mainz D, Stolte M, Labenz J, Malfertheiner P. Reversibility of *H. pylori* metronidazole resistance under partial anaerobic culture conditions does not predict *H. pylori* eradication. Gut 1999; 45 (Suppl. 111): A110.

Penston JG. The efficacy and safety of long-term maintenance treatment of duodenal ulcers with ranitidine. Scand J Gastroenterol. 1990; 25 (Suppl. 177): 42–51.

Perri F, Festa V, Clemente R, Quitadamo M, Andriulli A. Failure of standard triple therapies for *H. pylori* eradication in dyspeptic outpatients. Gut 1999; 45 (Suppl. 111): A113.

Perri F, Villani MR, Festa V, Quitadamo M, Andriulli A. Predictors of failure of Helicobacter pylori eradication with the standard 'Maastricht triple therapy'. Alimentary Pharmacology & Therapeutics 2001; 15 (7): 1023–1029.

Prewett, E, Bickley J, Owen RJ, Pounder RE. DNA patterns of *H. pylori* isolated from gastric antrum, body and duodenum. Gastroenterol 1992;102: 829–833.

Qasim, A, O'Morain CA. Review article: treatment of Helicobacter pylori infection and factors influencing eradication. Alimentary Pharmacology & Therapeutics 2002; 16 (Suppl. 1): 24–30.

Rauws EAJ, Tytgat GNJ. Cure of duodenal ulcer with eradication of *H. pylori*. Lancet 1990; 335: 1233–1235.

Reinbach DH, Cruckshank G, McColl KEL: Acute perforated duodenal ulcer is not associated with Helicobacter pylori infection. Gut 1993; 34: 1334–1337.

Reyrat J-M, Pelicic V, Papini E, Montecucco C, Rappuoli R, Telford JL. Towards deciphering the *Helicobacter pylori* cytotoxin. Mol Microbiol 1999; 34: 197–204.

Robles R, Parrilla P, Lujan JA, Torralba JA, Cifuentes J, Liron R, Pinero A. Short note: Long-term follow-up of bilateral truncal vagotomy and pyloroplasty for perforated duodenal ulcer. Br J Surg 1995; 82: 665.

Rodriquez LAG, Jick H. Risk of upper gastrointestinal bleeding and perforation associated with individual non-steroidal anti-inflammatorial drugs. Lancet 1994; 343: 769.

Roher HD, Imhof M, Goretzki PE, Ohmann C Ulcer surgery '96 — choice of methods in an emergency. Chirurg 1996; 67: 20–25.

Romero GM, Martinez DC, Grande L, Otero Fernandez MA, Vargas J, Castro FM. Intravenous eradication therapy for bleeding gastroduodenal ulcer associated with Helicobacter pylori infection. Rev Enferm Dig 2000; 24 (3): 299–305.

Rudi J, Kolb C, Maiwald M, Kuck D, Sieg A, Galle PR, Stremmel W. Diversity of *Helicobacter pylori vacA* and *cagA* genes and relationship to VacA and CagA protein expression, cytotoxin production, and associated diseases. J Clin Microbiol 1998; 36: 944–948.

Rudi J, Rudy A, Maiwald M, Kuck D, Sieg A, Stremmel W. Direct Determination of *Helicobacter pylori vacA* genotypes and *cagA* gene in gastric biopsies and relationship to gastrointestinal diseases. Am J Gastroenterol 1999; 94: 1525–1531.

Sarv J. Haavandtõve kirurgilise ravi aktuaalseid küsimusi. Nõukogude Eesti Tervishoid 1968;1:5–9.

Schein M, Gecelter G, Freinkel Z. APACHE II in emergency operations for perforated ulcers. Am J Surg 1990; 159: 309–313.

Schneider M. The quality of life in large American cities: Objective and subjective social indicators. Social Indicators Research 1973; 1: 495–509.

Sebastian M, Chandran VP, Elashaal YI, Sim AJ. Helicobacter pylori infection in perforated ulcer disease. Br J Surg 1995; 82: 360–362.

Sharma R, Organ CHJ, Hirvela ER, Henderson VJ. Clinical observation of the temporal association between crack cocaine and duodenal ulcer. Am J Surg 1997; 174 (6): 629–633.

Sheu BS, Chi CH, Yang HB, Jen CM, Lin XZ. A three day course of intravenous omeprazole plus antibiotics for H.pylori-positive bleeding duodenal ulcer. Hepato-Gastroenterol 1999; 46 (28): 2363–2371.

Shimoyama T, Fukuda S, Tanaka M, Mikami T, Saito Y, Munakata A. High prevalence of the *cagA*-positive *Helicobacter pylori* strains in Japanese asymptomatic patients and gastric cancer patients. Scand J Gastroenterol 1997; 32: 465–468.

Sibul U, Truve R. Mao resektsiooni ulatuse määramisest. Nõukogude Eesti Tervishoid 1968;1:9–12.

Sillakivi T, Aro H, Ustav M, Peetsalu M, Peetsalu A, Mikelsaar M. Diversity of *Helicobacter pylori* genotypes among Estonian and Russian patients with perforated peptic ulcer, living in Southern Estonia. FEMS Microbiol Letters 2001; 195: 29–33.

Sillakivi T, Lang A, Soplepmann J, Tein A, Peetsalu A. Incidence of perforated peptic ulcer correlated with suicide rate in Estonia in 1981–2000. Proceedings of the Eurosurgery 2002. Monduzzi Editore. Lisbon (Portugal), June 5–7, 2002: 199–204.

Sillakivi T, Peetsalu A. Perforeerunud peptilise haavandi riskifaktorid. Eesti Arst 2000; 12: 718–722.

Sillakivi T, Yang Q, Peetsalu A, Ohmann C. Perforated peptic ulcer: Is there a difference between Eastern Europe and Germany? Langenbeck`s Arch Surg 2000; 385: 344–349.

So JBY, Yam AW, Cheah WK, Kum CK, Goh PMY. Risk factors related to operative mortality and morbidity in patients undergoing emergency gastrectomy. Br J Surg 2000; 87: 1702–1707.

Sonnenberg A, Olson CA, Zhang J. The Effect of antibiotic therapy on bleeding from duodenal ulcer. Am J Gastroenterol 1999; 94: 950–954.

Soplepmann J, Peetsalu A, Peetsalu M, Tein A, Juhola M. Peptic ulcer haemmorrhage in Tartu county, Estonia: Epidemiology and mortality risk factors. Scand J Gastroenterol 1997; 32: 1195–1200.

Statistical Yearbook of Estonia 1999. Chapter: Labour market. The Statistical Office of Estonia, Tallinn, Estonia, 1999: 183.

Stewart DN, Wisner DM: Incidence of perforated ulcer: Effect of heavy air-raids. Lancet 1942; 1: 259.

Stolte M, Eidt S, Ritter M et al. Campylobacter pylori und Gastritis. Pathologe 1989;10: 21–26.

Strobel, S, Bereswill S, Balig P, Allgaier P, Sonntag H-G, Kist M. Identification and analysis of new *vacA* genotype variant of *Helicobacter pylori* in different patient groups in Germany. J Clin Microbiol 1998; 36: 1285–1289.

Suter M. Surgical treatment of perforated peptic ulcer. Is there a need for a change? Br J Surg 1995; 82: 1140–1141.

Svanes C, Espehaug B, Salvesen H, Søreide O, Svanes K A multifactorial analysis of factors related to lethality following treatment of perforated gastroduodenal ulcer 1935–85. Ann J Surg 1989; 209: 418–423.

Svanes C, Overbo K, Søreide O Ulcer bleeding and perforation: Non-steroidal anti-inflammatory drugs or Helicobacter pylori. Scand J Gastroenterol 1996; 220: 128–131.

Svanes C, Lie RT, Kvale G, Svanes K, Søreide O. Incidence of perforated ulcer in western Norway, 1935–1990: cohort- or period-dependent time trends? Am J Epidemiol 1995; 141: 836–44.

Svanes C, Salvesen H, Stangeland L, Svanes K, Søreide O: Perforated peptic ulcer over 56 years. Time trends in patients and disease characteristics. Gut 1993; 34:1666–1671.

Svanes C, Soreide JA, Skarstein A, Fevang BT, Bakke P, Vollset SE, Svanes K. Sooreide O. Smoking and ulcer perforation. Gut 1997; 41:177–180.

Svanes C: Trends in perforated peptic ulcer: incidence, etiology, treatment, and prognosis. World J Surg 2000; 24: 277–283.

The EUROGAST Study Group. Epidemiology of, and risk factors for, *Helicobacter pylori* infection among 3154 asymptomatic subjects in 17 populations. Gut 1993; 34: 1672–1676.

Tokumaru K, Kimura K, Saifuku K, Kojima T, Satoh K, Kihira K, Ido K. CagA and cytotoxity of Helicobacter pylori are not markers of peptic ulcer in Japanese patients. Helicobacter 1999; 4(1): 1–6.

Tokunaga J, Hata K, Ryo J, Kitaoka A, Tokuka A, Ohsumi K. Density of helicobacter pylori infection in patients with peptic ulcer perforation. J Am Coll Surg 1998; 186: 659–663.

Tonnesen T, Carlsen E. Perforated ulcer. Tidsskrift for Den Norske Laegeforening 2001; 121 (7): 790–792.

Towfight S, Chandler C, Hines OJ, McFadden DW. Outcomes from peptic ulcer surgery have not benefited from advances in medical therapy. Am Surg 2002; 68 (4): 385–389.

Tran T, Quandalle P. Treatment of perforated peptic ulcers by surgical suture followed by eradication of *Helicobacter pylori*. Annales de Chirurgie 2002; 127 (1): 32–34.

Treiber G, Wittig J, Ammon S, Walker S, van Doorn L-J, Klotz U. Clinical outcome and influencing factors of a new short-term quadruple therapy for Helicobacter pylori eradication: a randomized controlled trial (MACLOR study). Archives of Internal Medicine 2002; 162 (2): 153–160.

Truve R, Sibul U, Lutsuver A. Selektiivse vagotoomia tulemused kaksteistsõrmiksoole haavandtõve ravis. Nõukogude Eesti Tervishoid 1973; 1:3–7.

Tsugawa K, Koyanagi N, Hashizume M, Tomikawa M, Akahoshi K, Ayukawa K, Wada H, Tanoue K, Sugimachi K. The therapeutic strategies in performing emergency surgery for gastroduodenal ulcer perforation in 130 patients over 70 years of age. Hepato-Gastroenterol 2001: 48 (37): 156–162.

Tummuru MKR, Cover TL, Blaser MJ. Cloning and expression of a high-molecular-mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. Infect Immun 1993; 61: 1799–1809.

Tylor H. Perforated peptic ulcer: treated without operation. Lancet 1946; 2: 441.

Tylor JL, Zagari M, Murphy K, Freston JW. Pharmacoeconomic comparison of treatments for the eradication of H.pylori. Arch Int Med 1997; 157:87.

Tytgat GNJ. Treatment of Peptic Ulcer. Digestion 1998; 59:446–452.

Van Doorn LJ, Figueiredo C, Rossau R, Jannes G, van Asbroeck M, Sousa JC, Carneiro F, Quint WGV. Typing of *Helicobacter pylori vacA* gene and detection of *cagA* gene by PCR and reverse hybridization. J Clin Microbiol 1998; 36: 1271–1276.

Van Doorn LJ, Figueiredo C, Sanna R, Blaser MJ, Quint WGV. Distinct variants of *Helicobacter pylori* cagA are associated with vacA subtypes. J Clin Microbiol 1999; 37: 2306–2311.

Van Doorn LJ, Figueiredo C, Sanna R, Plaisier A, Schneebergen P, de Boer WA, Quint WGV. Clinical relevance of the *cagA, vacA*, and *iceA* status of *Helicobacter pylori*. Gastroenterol 1998;115: 58–66.

Van Doorn LJ, Schneeberger PM, Nouhan N, Plaisier AP, Quint WG, de Boer WA. Importance of *Helicobacter pylori cagA* and *vacA* status for the efficacy of antibiotic treatment. Gut 2000; 46: 321–326.

Vardja T, Peetsalu A, Peetsalu M, Soplepmann J, Tein A, Väli T. Surgical treatment of duodenal ulcer disease: A long-term follow-up study. Acta Medica Baltica 1996, 3: 256–263.

Veldhuyzen van Zanten SJO, Pollak PT, Best LM, Bezanson GS, Marrie T. Increasing incidence of Helicobacter pylori with age: continuous risk of infection in adults rather than cohort effect. J Infect Dis 1994; 169: 434–437.

Vorobjova T, Kisand K, Haukanõmm A, Maaroos H-I, Wadström T, Uibo R. The prevalence of Helicobacter pylori antibodies in a population from southern Estonia. J Gastroenterol Hepatol 1994; 6: 529–533.

Vorobjova T, Nilsson I, Kull K, Maaroos H-I, Covacci A, Wadström T, Uibo R. CagA protein seropsitivity in a random sample of adult population and gastric cancer patients in Estonia. Eur J Gastroenterol Hepatol 1998; 10: 41–46.

Väli T, Tamm A. Diagnostic criteria for lactose loading test after gastric surgery. Acta et commentationes Universitas Tartuensis, Tartu, 1994; 964:42–45.

Wakayama T, Ishizaki Y, Mitsusada M, Takahashi S, Wada T, Fukushima Y, Hattori H, Okuyama T, Funatsu H. Risk factors influencing the short-term results of gastroduodenal perforation. Surg Today 1994; 24: 681–687.

Walt R, Katchinski B, Logan R, Aschley J, Langman M. Rising frequency of perforation in elderly people in the United Kingdom. Lancet 1986; 1: 489–492.

Wang HJ, Kuo CH, Yeh AA, Chang PC, Wang WC. Vacuolating toxin production in clinical isolates of *Helicobacter pylori* with different *vacA* genotypes. J Infect Dis 1998; 178: 207–212.

Warren JR. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983; i: 1273.

Wasserman D, Värnik A: Increase in suicide among men in the Baltic countries. Lancet 1994; 343: 1504–1505.

Weir RD, Backett EM. Studies of the epidemiology of peptic ulcer in a rural community: prevalence and natural history of dyspepsia and peptic ulcer. Gut 1968; 9:75–83.

Welch CE, Rodkey GV, Gryska PV. A thousand operations for ulcer disease. Ann Surg 1986; 204: 454–467.

Wermeille J, Cunningham M, Dederding J-P, Girard L, Baumann R, Zelger G, Buri P, Metry J-M, Sitavanc R, Gallaz L, Merki H, Godin N. Failure of Helicobacter pylori eradication: is poor compliance the main cause? Gastroenterologie Clinique et Biologique 2002; 26 (3): 216–219.

Wermeille J, Zelger G, Cunningham M. The eradication regimens of *Helicobacter pylori*. Pharmacy World & Science 1998; 20 (1): 1–17.

SUMMARY IN ESTONIAN

Perforeerunud peptiline haavand Eestis: epidemioloogia, riskifaktorid ning seosed *Helicobacter pyloriga*

Perforeerunud peptilist haavandit (PPH) kirjeldas esmakordselt Crisp juba 1843 aastal. Sellest ajast on aga PPH epidemioloogias toimunud suured muutused. Viimastel aastakümnetel on arenenud riikides PPH esinemissagedus langenud noortel patsientidel ja meestel, kuid on tõusnud eakamatel inimestel ja naistel. Seejuures on tõusnud PPH patsientide keskmine vanus, naiste osakaal, mitte-steroidsete põletikuvastaste ainete tarvitajate osakaal ning kaasuvate haigustega patsientide arv.

Kuigi plaaniline kirurgiline ravi haavandtõve puhul on kogu maailmas oluliselt langenud tänu uute võimsate ravimite kasutusele võtmisele, pole haavandtõve tüsistuste kirurgilise ravi vajadus vähenenud. Vastavalt Sotsiaalministeeriumi andmetele on Eestis PPH tõttu teostatud erakorraliste operatsioonide arv märgatavalt kasvanud alates 1991 aastast.

PPH kirurgilises ravis on toimunud muutused mittedefinitiivsete, haavandi patogeneetilisi mehhanisme mittemõjutavate operatsioonide kasuks. Niisugustele ravitaktika muutustele on kõige olulisemat mõju avaldanud *Helicobacter pylori* eradikatsiooni postoperatiivne rakendamine. Samas erinevalt näiteks haavandtõve teisest tüsistusest, haavandi verejooksust, pole PPH puhul selle ravitaktika kaugtulemused erinevate uurijate poolt seni piisavalt tõestatud. Ka *H. pylori* enese roll haavandi perforeerumisel pole üheselt määratletav. Küll on aga kaugtulemuste võrdlemine näidanud, et definitiivsete operatsioonimeetodite kasutamisel on tulemused paremad kui mittedefinitiivsete meetodite kasutamisel.

Vaatamata meditsiini edusammudele pole ilmnenud ka PPH ravitulemuste paranemist, kusjuures surevuse näitajad püsivad endiselt kõrged, seda eriti vanemate patsientide puhul. Surevus on seotud peamiselt patsientide vanuse, kaasuvate haiguste esinemise ning hilinenud raviga. Viimastes uuringutes pole leitud tõestust, et operatsioonimeetodi valik ise mõjutaks surevuse näitajaid.

H. pylori esinemissagedus Eestis (kuni 87% populatsioonist) on oluliselt kõrgem kui arenenud riikides (umbes 50%). PPH patsientide puhul kõigub *H. pylori* esinemissagedus maailmas väga suurtes piirides (50–97%).

Nüüdseks on üha enam töid suunatud ka *H. pylori* erinevate tüvede ja nende virulentsusmarkerite uurimisele, et selgitada seoseid nende faktorite ja erinevate maohaiguste tekke vahel. Seni on erinevates uuringutes leitud seosed sageli vastuolulised.

Uurimistöö eesmärgid ja ülesanded

Arvestades asjaolu, et haavandtõve eluohtliku tüsistuse, PPH, esinemissagedus Eestis on järsult tõusnud alates 1991 aastast ja selle haiguse epidemioloogiat,

aga samuti seoseid PPH ja *H. pylori* vahel pole Eestis varem uuritud, püstitati antud uurimistöös järgmised eesmärgid:

1. Uurida PPH epidemioloogilist olukorda ja muutusi Tartu piirkonnas 20 aastase perioodi jooksul (1981–2000).
2. Võrrelda Ida-Euroopa (s.h. Eesti) PPH patsientide vanuselist ja soolist koosseisu, tüsistusi ja ravitulemusi Lääne Euroopa (Saksamaa) PPH patsientide vastavate andmetega.
1. Hinnata jätkuvalt kõrge PPH esinemissageduse püsimise võimalikke põhjusi (riskifaktoreid) Eestis.
2. Hinnata riskifaktoreid, mis mõjutavad ravitulemust ja suremust PPH patsientide puhul, eriti seost ravitulemuse ja kasutatud operatsioonimeetodi vahel.
3. Määrata kindlaks *H. pylori* infektsiooni esinemine ja võrrelda selle bakteri genoomi varieeruvust proovides, mis on saadud PPH patsientidelt, kes elavad Lõuna-Eestis, kuid kuuluvad erinevatesse rahvustesse (eestlased ja venelased).
4. Võrrelda laialdaselt tuntud *H. pylori* virulentsusmarkerite, *cagA* ja *vacA*, jaotumust erinevate maohaigustega (krooniline gastriit, peptiline haavand ja PPH) Lõuna-Eesti patsientidel.
5. Hinnata erinevate postoperatiivsete *H. pylori* eradikatsioonile suunatud raviskeemide efektiivsust Eesti PPH patsientidel.

Uurimistulemused peaksid ühtlasi kaasa aitama teaduspõhiste, Eesti oludele sobivate efektiivsete PPH ravijuhiste väljatöötamisele.

Uuritav materjal ja meetodid

Antud uuringusse kaasati kokku 780 patsienti. Nende hulgast 504 PPH patsienti hospitaliseeriti TÜ Kirurgiakliinikusse aastatel 1978–2000, kellest 129 osalesid prospektiivsetes uuringutes. Erinevate alauuringute puhul esines uurimisgruppide oluline kattumine, vastavatesse analüüsidesse hõlmatud patsientide arv kõikus vahemikus 6 kuni 426. Lisaks nendele uuriti 134 PPH patsienti 11 Ida-Euroopa keskusest ja 37 Saksamaalt pärit PPH patsienti. Tüsistunud haavandtõvega patsientide kõrval lülitati uuringusse ka 36 kroonilise gastriidi ja 69 mittetüsistunud haavandtõvega patsienti Lõuna-Eesti regioonist.

Kahes retrospektiivses uuringus, mis käsitlesid PPH epidemioloogiat Tartu piirkonnas aastatel 1981–2000 ja surevuse riskifaktoreid kirurgiliselt ravitud PPH puhul, uuriti algmaterjalina läbi 1978–2000 aasta haiguslood ning vajadusel ka operatsiooniraamatud ja haigete hospitaliseerimise registreerimisraamatud.

Prospektiivsed uuringud viidi läbi aastatel 1995–2000. Seejuures kasutati nii spetsiaalseid uurimisprotokolle haigete põhjaliku küsitluse, kliinilise uuringu, ravitulemuse jälgimise ja endoskoopiate läbiviimise kohta kui ka histoloogilisi

ja molekulaarseid (polümeraasahelreaktsioon) uuringuid *H. pylori* ja tema virulentsusmarkerite määramiseks.

H. pylori eradikatsiooni üritati postoperatiivselt nii klassikaliste suukaudsete raviskeemide kui ka modifitseeritud, sealhulgas intravenoossete, raviskeemide abil. Eradikatsiooni läbinud patsiendid hõlmati 2 kuu möödumisel ravist järelkontrollidesse, mille käigus kasutati endoskoopilisi, histoloogilisi ja molekulaarseid uuringuid.

Kõikidel juhtudel sisestati andmed enne analüüsimist arvutiandmebaasidesse.

Uurimistööst tulenevad järeldused

1. Järsk, 2,7 kordne PPH esinemissageduse tõus leidis aset Tartu piirkonnas perioodil 1991–2000 võrreldes perioodiga 1981–1990, kusjuures PPH iga-aastased esinemissagedused aastatel 1981–2000 korreleerusid iga-aastaste suitsiidikordajatega samal perioodil. Perioodide võrdlusel ei täheldatud olulisi muutusi PPH patsientide vanuselises ega soolises koosseisus ega ka surevuses. PPH keskmine esinemissagedus perioodil 1991–2000 (20.0) ületab vähemalt 2–3 korda arenenud riikide vastava näitaja.
2. Uuritud Eesti PPH patsiendid olid nooremad, naiste osakaal väiksem ja definitiivsete operatsioonimeetodite kasutamine sagedasem kui Saksamaal või teistes arenenud riikides läbiviidud uuringute puhul. Samas Eesti PPH patsientide vanuseline ja sooline jaotumus, kirurgiliste meetodite kasutamine ja suremus olid sarnased Ida-Euroopas läbiviidud uuringute vastavate näitajatega.
3. PPH kõrget esinemissagedust Eestis alates 1991. aastast võib seostada kõrgenenud (sotsiaalse) stressi ja suitsetamisega ning kaasaegse adekvaatse medikamentoosse ravi mittekasutamisega haavandtõvega haigetel. Et vähendada peptilise haavandi eluohtliku tüsistuse, perforatsiooni, teket, võiksid meetmed olukorra parandamiseks olla suunatud nii arstkonnale kui ka selgitustöö tõhustamisele elanikkonna seas.
4. Patsientide kõrge iga ja kaasuvate haiguste esinemine osutusid iseseisvateks surevust mõjutavateks faktoriteks PPH patsientide kirurgilise ravi korral. Vanus ≥ 60 aastat ja ≥12 tunni möödumine perforatsioonist enne ravi alustamist olid olulisteks tüsistuste teket määravateks faktoriteks. Tüsistused ja suremus ei sõltunud sellest, kas kasutati definitiivseid või mittedefinitiivseid operatsioonimeetodeid.
5. *H. pylori* infektsioon esines 97% Eesti PPH patsientidest. Lõuna-Eesti PPH patsientide *H pylori* tüvede puhul ilmnesid geograafilised ja etnilised erinevused: vacA geeni alatüüpide jaotumus oli statistiliselt erinev eesti ja vene rahvusest patsientidelt isoleeritud proovides; kusjuures *vacA* geeni s1b alatüüpi, mis on mõnedes riikides laialdaselt levinud, ei leitud meie patsientidelt isoleeritud bakteritüvedel. Antud tulemused võivad osutuda

tähtsaks erinevate etniliste gruppide puhul uute PPH ravi- ja profülaktiliste strateegiate väljatöötamisel.

6. Erinevate maohaigustega Lõuna-Eesti patsientidelt isoleeritud *H. pylori* tüvedele on iseloomulik kõrge (87%) cagA geeni esinemissagedus. *H. pylori* tüvede jaotumus virulentsusmarkerite, cagA ja vacA genotüübi, põhjal ei erinenud statistiliselt kroonilise gastriidi, mittetüsistunud haavandtõve ega PPH puhul.
7. Kõik käesolevas töös *H. pylori* eradikatsiooniks kasutatud raviskeemid osutusid PPH puhul ebaefektiivseks, mis seab kahtluse alla niisugusel kujul ravitaktika kasutamise Eesti patsientidel. Täpsem ebaedu põhjuste selgitamine ning Eesti oludele sobiva ravitaktika väljatöötamine nõuab jätkuvaid uuringuid.

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PUBLICATIONS

I

Sillakivi T, Lang A, Soplepmann J, Tein A, Peetsalu A. Incidence of perforated peptic ulcer correlated with suicide rate in Estonia in 1981–2000. Proceedings of the Eurosurgery 2002. Monduzzi Editore. Lisbon (Portugal), June 5–7, 2002: 199–204.

Incidence of Perforated Peptic Ulcer Correlated with Suicide Rate in Estonia in 1981-2000

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Summary

Introduction: The aim of this study was to assess the characteristics of perforated peptic ulcer (PPU) in Tartu county, Estonia, in 1981-2000.

Materials and Methods: All 426 patients with PPU from this defined area, admitted during 1981-2000, were analysed in a retrospective study.

Results: Comparison of the periods 1981-90 and 1991-2000 revealed a sharp increase in PPU incidence from mean 7.4 to 20.0 per 100,000/year ($p<0.001$). The annual incidences of PPU were significantly correlated with the annual suicide rates for 1981-2000. PPU patients' mean age and sex distribution did not change. The mean age was 46.7 years, being 62.2 years for females and 42.3 years for males ($p<0.001$). Of the patients 22.3% (95/426) were female and 30.4% (112/369) had a history of ulcer disease before perforation. PPU mortality remained fairly stable, 6.0% (7/117) and 5.8% (18/309) in 1981-90 and 1991-2000, respectively (p=ns).

Conclusions: A pronounced increase in PPU incidence occurred in Tartu county without significant changes in patients' mean age, sex distribution and outcome. The annual PPU and suicide rates were correlated.

Introduction

In recent decades the epidemiology of perforated peptic ulcer (PPU) has undergone considerable changes in the Western countries. PPU inci-

dence has decreased in young patients and males, but increased in elderly patients and females (1). Mean age of patients at the time of perforation has risen by 10-20 years (2, 3).

According to the reports of the Ministry of Social Affairs of Estonia increase in the incidence of PPU in Estonia has been considerable since 1991. The aim of this study was to examine the characteristics of PPU in a defined area in the southern part of Estonia, Tartu county, in the 20-year period from 1981 to 2000.

PPU has been associated with social stress (4), and suicide rate has been pointed out as an indirect stress parameter (5). Since not only PPU incidence, but also suicide rate was increasing rapidly in Estonia from 1991 (6), we aimed to evaluate whether the PPU and suicide rates were statistically correlated for 1981-2000.

Materials and Methods

All 426 patients with PPU from the catchment area of Tartu county between 1981 and 2000 were included in a retrospective study. The estimated mean population of Tartu county was 156,993 (standard error ±896) throughout the 20-year study period according to data published by the Statistical Office of Estonia. No considerable changes have taken place in the age or sex distribution of the population in this period.

Primary data for identification of patients were obtained from the computerised database of the hospital according to the discharge diagnosis. Further, case histories and logbooks of the operating theatre were studied. No changes took place in disease classification during the studied period.

The diagnosis of PPU was confirmed in 426 patients - in 398 patients on operation, in 4 patients on autopsy and 4 conservatively managed patients (with typical clinical signs, pneumoperitoneum on X-ray and endoscopically verified ulcer disease) were also included. Ulcers were classified into gastric (GU) and duodenal ulcers (DU), while DU comprised prepyloric (within 0.5-2.0 cm from the pyloric ring), pyloric and bulbar ulcers as described by Horowitz (7).

To compare the study periods, the patients were divided into two groups according to decades: period I (1981-90) and period II (1991-2000). The correlation between the annual incidences of PPU in Tartu county and the suicide rates in Estonia for 1981-2000 was evaluated using the Pearson correlation test. The data of the suicide rates in Estonia were drawn from the database of the Swedish-Estonian Institute of Suicidology. Mean values are presented as the mean ± standard error of the mean and were compared with the use of Student's t-Test. Absolute figures were compared by using chi-square test with continuity correction. Differences were considered statistically significant for p values less than 0.05.

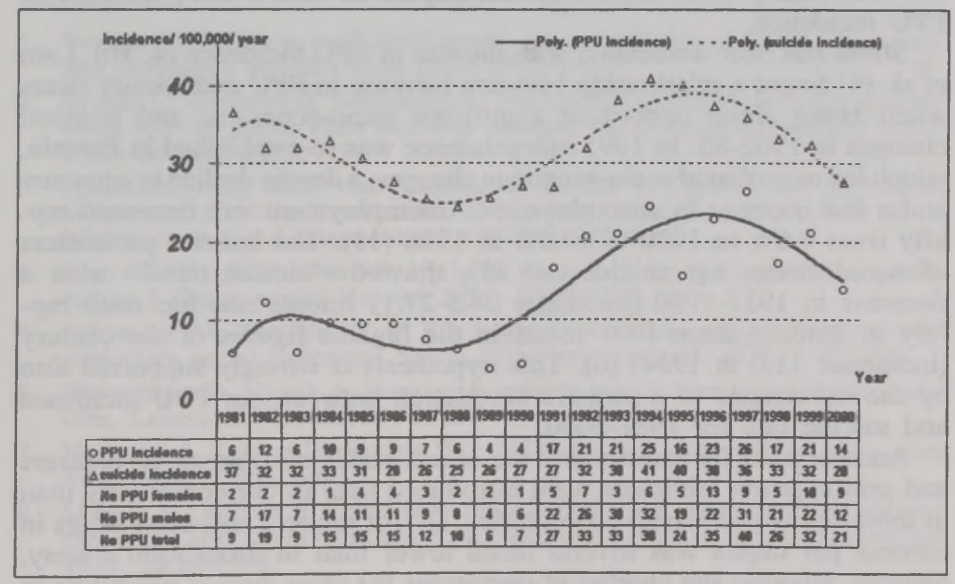
Results

The incidence of PPU remained between 4 and 12 (mean 7.4±0.9) per 100,000 per year in period I (Figure 1). In period II there occurred a sharp increase in incidence up to 26, which did not drop below 14 (mean 20.0±1.3) ($p<0.001$). The Pearson correlation test revealed a statistically significant correlation between the annual incidences of PPU and the suicide rates in the period 1981-2000 ($r=0.633$, $p=0.0027$) (Figure 1).

An increasing tendency of PPU incidence was similar for both of male and female patients while the female:male ratio (1:4.1 in period I and 1:3.3 in period II) did not reveal any significant changes (p=ns). Mean age was almost the same, 47.4±1.7 years in 1981-90 and 46.4±1.1 years in 1991-2000 (p=ns). The mean age of women, 62.2±2.0 years, exceeded significantly that of men 42.3±0.9 ($p<0.001$). Mean age for GU perforations was 56.9±2.3 years and for DU perforations 45.0±1.0 years ($p<0.001$).

GU perforations accounted for 11.1% (13/113) and 11.9% (37/304) of all perforations in periods I and II, respectively (p=ns). Of the patients 30.4% (112/369) had a history of confirmed ulcer disease before perforation and 23.4% (94/402) had concomitant diseases.

Twenty-five patients (5.9%) of 426 with PPU died during 1981-2000. Mortality remained almost the same, 6.0% (7/117) in period I and 5.8%



	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
○ PPU incidence	6	12	6	10	9	9	7	6	4	4	17	21	21	25	16	23	26	17	21	14
△ suicide incidence	37	32	32	33	31	28	26	25	26	27	27	32	38	41	40	38	36	33	32	28
No PPU females	2	2	2	1	4	4	3	2	2	1	5	7	3	6	5	13	9	5	10	9
No PPU males	7	17	7	14	11	11	9	8	4	6	22	26	38	32	19	22	31	21	22	12
No PPU total	9	19	9	15	15	15	12	10	6	7	27	33	33	38	24	35	40	26	32	21

Fig. 1. Incidences per 100,000 per year and the polynomial curves (calculated from the formula $y=b+c_1x+c_2x^2+c_3x^3$) of perforated peptic ulcer (PPU) in Tartu county and of suicide in Estonia; number of PPU (females, males, total) in Tartu county in 1981-2000.

(18/309) in period II (p=ns). Mortality for females was 15.8% versus 3.0% for males (p<0.001). However, it revealed no significant difference when different age groups were compared: 24.2% (8/33) for males and 27.1% (13/48) for females, both aged ≥65, and 0.7% (2/298) for males and 4.3% (2/47) for females, both aged <65. The mean age of patients who died, 74.2±2.5 years, exceeded significantly that of patients who survived, 45.0±0.9 years (p<0.001).

Discussion

A sharp, 2.7-fold increase in PPU incidence was noted in Tartu in 1991-2000 compared with 1981-1990, while the same pattern is characteristic of the whole of Estonia. The PPU incidence of 20.0 for 1991-2000 is at least 2-3 times as high as that reported from the Western countries, 2.3-9.2 (3, 8). There were no considerable changes in our patients' mean age and sex distribution throughout the 20 years studied. However, the mean age of our patients was considerably lower (46.7 years vs 53-63 years) and the proportion of female patients (22.3% versus about 40-60%) lower compared with the figures given in most Western studies (2, 3, 8, 9).

We consider that factors as social stress, low anti-ulcer drug usage and possibly factors as smoking habits and high *H. pylori* infection rates in our community could have a certain impact on such a sharp increase of PPU incidence.

Stress has been associated with the rise of PPU incidence (4, 10). Lam et al. (4) found a relationship between increase in PPU and society stress when Hong Kong underwent significant socio-economic and political changes in 1962-85. In 1991 independence was re-established in Estonia, which led to profound socio-economic changes: a drastic decline in economy and a fast increase in unemployment. Unemployment rate increased rapidly from 0.6% in 1990 to 10.0% in 1996 (11). The indirect parameters of social stress, e.g. suicide rate (5), showed a similar trend - after a decrease in 1987-1990 (incidence 24.5-27.1) suicide rate has risen rapidly in Estonia since 1991 to attain the highest figures of the century (incidence 41.0 in 1994) (6). This hypothesis is strongly supported also by the occurrence of a positive correlation between our PPU incidence and suicide rate for 1981-2000.

Another point for discussion is use of anti-peptic-ulcer drugs. H_2 blockers and proton pump inhibitors were introduced later in our community than in most developed European countries. Use of modern anti-ulcer drugs in Estonia per capita was several times lower than in Stockholm county, Sweden, whereas the number of operations for ulcer disease per capita in Estonia was almost 20 times higher in 1993-95 (12). Smoking plays an important role in ulcer perforation, especially in younger patients (13). Although respective data were not available for our studied patients, in

our ongoing prospective study over 80% of PPU patients from the same area were daily smokers in 1997-2000 (unpublished data). At the same time, the proportion of daily smokers in general population was only 29-36% in the period 1990-2000 (14).

The role of the important aetiological factor of peptic ulcer, *H. pylori*, has remained controversial in PPU (15, 16). The prevalence of *H. pylori* infection in Estonia is higher than in the Western countries, affecting 78-87% of population in Southern Estonia (17). In a prospective study genotyping *H.pylori* strains, the bacteria were detected in 98% of the PPU patients from the same area studied in 1997-99 (18).

Mortality of PPU patients in our study (mean 5.9% for 20 years) was considerably lower than in most recent studies (3, 9). Also, mortality rates did not change during the study period, differently from increasing trends established in Western studies (2, 9). The lower mortality can be related mainly to the younger structure of our PPU patient sample which did not reveal any increasing age trend.

In conclusion, a significant increase in PPU incidence occurred in Tartu county since 1991, while the annual incidences of PPU were statistically correlated with the suicide rates for the period 1981-2000. At the same time, the mean age and sex distribution of PPU patients as well as outcome did not reveal any change.

References

1. Svanes C: Trends in perforated peptic ulcer: incidence, etiology, treatment, and prognosis. World J Surg 2000; 24:277-283.
2. Svanes C, Salvesen H, Stangeland L, Svanes K, Søreide O: Perforated peptic ulcer over 56 years. Time trends in patients and disease characteristics. Gut 1993; 34:1666-1671.
3. Hermansson M, Stael von Holstein C, Zilling T: Peptic ulcer perforation before and after the introduction of H_2-receptor blockers and proton pump inhibitors. Scand J Gastroenterol 1997; 32:523-529.
4. Lam SK, Hui WM, Shiu LP, Ng MM: Society stress and peptic ulcer perforation. J Gastroenterol Hepatol 1995; 10:570-576.
5. Schneider M: The quality of life in large American cities: Objective and subjective social indicators. Social Indicators Research 1973; 1:495-509.
6. Wasserman D, Värnik A: Increase in suicide among men in the Baltic countries. Lancet 1994; 343:1504-1505.
7. Horowitz J, Kukora JS, Ritchie WP: All perforated ulcers are not alike. Ann Surg 1989; 209:693-696.
8. Makela J, Laitinen S, Kairaluoma MI: Complications of peptic ulcer disease before and after the introduction of H_2-receptor antagonists. Hepatogastroenterol 1992; 39:144-148.
9. Gunshefski L, Flancbaum L, Brolin RE, Frankel A: Changing patterns in perforated peptic ulcer disease. Am Surg 1990; 56:270-274.
10. Stewart DN, Wisner DM: Incidence of perforated ulcer: Effect of heavy air-raids. Lancet 1942; 1:259.

11. Statistical Yearbook of Estonia 1999. Chapter: Labour market. The Statistical Office of Estonia, Tallinn, Estonia, 1999: 183.
12. Kiivet R-A, Bergman U, Sjostedt S, Sjoqvist F: Ulcer surgery in Estonia, a consequence of drug delay? Lancet 1998; 351:146.
13. Svanes C, Soreide JA, Skarstein A, Fevang BT, Bakke P, Vollset SE, Svanes K. Sooreide O: Smoking and ulcer perforation. Gut 1997; 41:177-180.
14. Kasmel A, Lipand A, Markina A, Kasmel K: Health behaviour among Estonian adult population, spring 2000. OÜ Dada AD, Tallinn, Estonia, 2001. Table 30.B.
15. Sebastian M, Chandran VP, Elashaal YI, Sim AJ: Helicobacter pylori infection in perforated ulcer disease. Br J Surg 1995; 82:360-362.
16. Reinbach DH, Cruckshank G, McColl KEL: Acute perforated duodenal ulcer is not associated with Helicobacter pylori infection. Gut 1993; 34:1334-1337.
17. Maaroos H-I: Helicobacter pylori infection in Estonian population: is it a health problem? Ann Med 1995; 27:613-616.
18. Sillakivi T, Aro H, Ustav M, Peetsalu M, Peetsalu A, Mikelsaar M: Diversity of *Helicobacter pylori* genotypes among Estonian and Russian patients with perforated peptic ulcer, living in Southern Estonia. FEMS Microbiol Letters 2001; 195:29-33.

II

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Original Paper

Evaluation of Risk Factors for Mortality in Surgically Treated Perforated Peptic Ulcer

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ABSTRACT

Background/Aims: The aim of our study was to evaluate complications and possible risk factors for mortality in perforated peptic ulcer patients with a special reference to the fact whether definitive or non-definitive operation was performed.

Methodology: All 394 patients (mean age: 45.5 years; range: 15-93) from Tartu county hospitalized for PPU at Tartu University Clinic in the period 1978-97 were included in a retrospective study.

Results: Twenty-two patients (5.6%) of 394 died. In 73 patients 93 concomitant diseases (mortality 19.2%) and in 81 patients 114 complications were observed. There were 245 non-definitive operations and 141 definitive operations with a mortality rate of 7.3% and 1.4%, respectively. Univariate logistic regression analysis of 386 operatively treated patients revealed that age ≥65 years, concomitant diseases, treatment delay ≥12 hours, female sex and non-definitive operations were significantly associated with mortality. However, multivariate analysis showed that only age ≥65 years and concomitant diseases were independent predictors of mortality.

Conclusions: Patients' high age and presence of concomitant diseases were related to lethal outcome after surgical procedure performed for perforated peptic ulcer. The result did not depend on the fact whether definitive or non-definitive operation was applied.

KEY WORDS: Perforated peptic ulcer; Surgery; Mortality risk factors

ABBREVIATIONS: Perforated Peptic Ulcer (PPU); Non-Steroidal Anti-Inflammatory Drugs (NSAID); Odds Ratio (OR); Confidence Interval (CI)

INTRODUCTION

There has been substantial decline in elective operations for peptic ulcer disease during recent decades following the introduction of H2-receptor antagonists (1). However, surgery for peptic ulcer complications, including perforations, has not decreased despite the use of new powerful drugs (1-3). Moreover, with increasing age and concomitant diseases in perforated peptic ulcer (PPU) patients (4-6), high complication and mortality rates have remained a challenging problem for the surgeon. Several studies discussing safety and risk factors of definitive surgery in emergency situation have established that definitive surgery in emergency situation is as safe as non-definitive (6-8), even in high risk patients (9,10). With a new understanding of peptic ulcer pathogenesis and advent of new powerful medicines, simple operations as ulcer suturation, including laparoscopic management, are increasingly advocated for optimal surgical treatment in recent years (11-14). However, there can be indications for definitive surgery as well, at least in a select group of patients (8). The aim of our study was to evaluate complications, outcome and possible risk factors for mortality in PPU patients with a special reference to the type of operation performed.

METHODOLOGY

All patients hospitalized for PPU at the Tartu University Clinic from Tartu county between 1 January 1978 and 31 December 1997 were included in a retrospective study. Case reports or, in case of missing data, operation theatre logbooks of these 394 patients (mean age: 45.5 years; range: 15-93, 17.3% of patients ≥65 years) were reviewed. The following data were recorded and entered into the database: patients' age and sex, previous ulcer history, concomitant diseases, shock on admission (systolic blood pressure <100mm Hg), treatment delay, ulcer location, operation, complications and outcome. There were 313 males and 73 females; according to ulcer location, the perforations were classified as follows: 44 gastric and 337 duodenal (including prepyloric, i.e., within 0.5-2.0cm of the pyloric ring, pyloric and bulbar) ulcers. Mortality was assessed during the time that the patient remained in hospital irrespective of the 30-day period. We considered it impossible to evaluate data on non-steroidal anti-inflammatory drugs (NSAID) usage and smoking on the basis of these documents.

During the 20-year study period, a significantly increasing trend of PPU incidence was noted, while the proportion of definitive operations increased substantially as well (unpublished data).

Evaluation of possible risk factors for mortality was carried out on 386 out of 394 (i.e., only surgically treated) patients using univariate analysis. The analysis involving age, sex and operative method was performed on all patients; the other parameters (treatment delay, location of ulcer, general/local peritonitis, concomitant diseases, shock on admission, previous ulcer history) were evaluated using the data that were available. Non-operatively treated patients were excluded from risk stratification. Further multivariate logistic regression was performed to analyze mortality risk factors. Only significant factors (five) from univariate analysis were included in the model. For other statistical analyses χ^2 method was used. Statistical calculations were made

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TABLE 1 Distribution of 114 Complications in 81 PPU Patients

Complication	No.of complications/ deaths
Wound infection	**26/2**
Intraabdominal complications:	**18/7**
Dehiscence, abscess	6/3
Anastomositis	5/3
Bleeding	4/ -
Pylorostenosis	2/1
Reperforation	1/ -
General complications:	**70/22**
Cardiac insufficiency/failure	19/3
Pneumonia	11/2
Respiratory failure, pulmonary embolism	5/3
Psychosis	7/1
Multiple Organ Failure	7/7
Renal failure	3/1
Sepsis	7/6
Others	11/ -

TABLE 2 Distribution of 93 Concomitant Diseases in 73 PPU Patients

	Patients aged <65years n=320	Patients aged ≥65years n=66
No of patients	40	33
Diseases:		
Cardiac insufficiency	6	23
Pulmonary	7	4
Tuberculosis in anamnesis	6	2
Rheumatic	4	7
Gynecological	5	-
Malignancy	2	2
Mental disorders	4	-
Diabetes mellitus	2	2
Hepatic	3	1
Neurological	4	-
Others	7	2
Total	50	43

TABLE 3 Operations and Outcome in 386 PPU Patients

	All patients (No. of deaths)	Gastric ulcer (No. of deaths)	Duodenal ulcer (No. of deaths)
Non-definitive operations			
Ulcer suturation	183* (15)	35 (3)	143 (12)
Ulcer excision ±PP	62 (3)	0	62 (3)
Total	245* (18)	35 (3)	205 (15)
Definitive operations			
TV+ ulcer excision+PP	104	0	104
SPV+ulcer excision±PP	9	0	9
SPV+suturation	1	0	1
Antrumectomy BI, BII	24 (2)	9 (1)	15 (1)
TV+ antrumectomy BI	3	0	3
Total	141 (2)	9 (1)	132 (1)

* in 5 patients the exact location of ulcer was unknown
PP: pyloroplasty; TV: truncal vagotomy; SPV: selective proximal vagotomy; BI and BII: Billroth I and II resection

with Stata statistical software.

RESULTS

Of the 394 patients 22 died yielding an overall mortality rate of 5.6%. In two patients perforation was diagnosed only on postmortem autopsy, and 20 patients who died had been operated. The mean age of the patients who died (71.8 years) exceeded significantly that of the patients who survived (43.9 years) (P=0.0001). Mortality of female patients (14.7%) exceeded significantly that of males (3.4%) (P=0.0004), however, the mean age of all women, 59.4 years, was significantly higher than that of men, 42.2 years (P=0.0001).

A total of 114 complications were observed in 81 patients: 54 patients had one complication, 22 patients had two, 4 patients had three and 1 patient had four complications. There were 26 wound infections, 18 intraabdominal and 70 general complications (**Table 1**). The commonest complications in the patients who died were multiple organ failure (7 patients) and sepsis (5 patients).

Seventy-three patients had 93 concomitant medical illnesses on admission. The most frequent concomitant diseases were cardiac (29), pulmonary (11) and rheumatic (11) illnesses (**Table 2**). In 40 patients aged <65 years, 50 concomitant diseases (mortality 5.0%, 2 of 40) and in 33 patients aged ≥65 years, 43 concomitant diseases (mortality 36.4%, 12 of 33) were recorded. Mortality of patients with concomitant diseases, 19.2% (14 of 73), exceeded significantly that of patients without them, 0.4% (1 of 277) (P<0.0001).

The operations performed on 386 patients are given in **Table 3**. In 245 patients non-definitive and in 141 patients definitive operations were used with a mortality rate of 7.3% (18/245) and 1.4% (2/141), respectively. Fifteen deaths occurred in patients treated with ulcer suturation, three deaths occurred in the ulcer excision and pyloroplasty group and only two deaths were registered in patients treated with definitive operation (partial gastrectomy). **Table 4** presents comparison of two patient groups where different operation methods were employed. In the group of non-definitive operations, patients were significantly older, had longer treatment delay, higher percentage of concomitant diseases and higher proportion of gastric ulcers. Six patients had intraoperative complications: there were 3 splenic, 2 bowel and 2 bile duct injuries.

The results of the evaluation of risk factors for mortality by univariate logistic analysis are given in **Table 5**. Age ≥65 years, female sex, treatment delay ≥12 hours, concomitant diseases and non-definitive operation increased mortality risk on the basis of univariate analysis. Inclusion of all these five variables in a multivariate regression model revealed that only patients' age ≥65 years (OR 13.9, CI 2.83-68.2, P=0.01) and concomitant diseases (OR 10.7, CI 2.69-42.4, P=0.01) were significant (independent) predictors of mortality.

DISCUSSION

Optimal management of patients with PPU is still a matter of discussion. After a significant decrease in elective ulcer surgery since the 1970s (1,15), the number of operations for peptic ulcer disease complications has remained almost stable (1-3,16,17). Decrease in mortality rates, from 20-30% in the 1930s-1940s (18) to about 5-10% in the 1970s (5,7,19), has not been observed in the

TABLE 4 Patient Characteristics According to Operation Type

Factor	Non-definitive op.	Definitive op.	χ^2 test, *P* value
No. of patients	245	141	
Mean age (years)	47.2	42.5	$P<0.05$
Male:female ratio	196:49	117:24	NS
Previous ulcer history	54 (27.4%)	46 (38%)	NS
Treatment delay <12 hours / ≥12 hours	163:68 (70.6%:29.4%)	119:16 (88.2%:11.8%)	$P<0.001$
Concomitant disease	54 (24.7%)	18 (14.1%)	$P<0.05$
Gastric/duodenal ulcer	35:205 (14.6%:85.4%)	9:132 (6.4%:93.6%)	$P<0.05$
Perforation size <6mm/ ≥6mm	164:49 (77%:23%)	99:31 (76.2%:23.8%)	NS

TABLE 5 Results of Evaluation of Possible Risk Factors for Mortality on Univariate logistic Regression Analysis

Factor	No. of patients	Mortality N	(%)	OR	CI 95%	*P* value
Age <65: ≥65 years	320:66	3:17	(0.9%:25.8%)	36.7	10.4-129.7	$P<0.001$
Male:female	313:73	10:10	(3.2%:13.7%)	4.8	1.9-12.0	$P<0.01$
Treatment delay <12:≥12 hours	303:81	8:12	(2.6%:14.8%)	6.4	2.5-16.2	$P<0.001$
Non-definitive: definitive operation	245:141	18:2	(7.3%:1.4%)	5.5	1.3-24.1	$P<0.05$
Duodenal:gastric ulcer	337:44	16:4	(4.7%:9.1%)	2.0	0.6-6.3	NS
General:localized peritonitis*	333:42	20:0	(6.0%:0%)	2.7	0.4-16.0	NS
Concomitant disease yes:no	72:277	13:2	(18.1%:0.7%)	30.3	6.7-137.8	$P<0.001$
Shock yes:no	17:320	1:14	(5.9%:4.4%)	1.4	0.2-11.0	NS
Ulcer history yes:no	101:255	2:15	(2.0%:6.0%)	0.3	0.7-1.3	NS

* to calculate OR, value 0 was substituted for by value 1.
OR: odds ratio; CI: confidence interval

1980s-1990s (5,20). Moreover, mortality figures as high as 17-31% can be found in literature (4,9,21,22). This trend can be explained mainly by changes in the structure of PPU patient population. During recent decades patients' mean age has increased by about 20 years (5,23,24); the proportion of female patients makes up nearly one half or even exceeds it (4-6,8,21); older patients have concomitant diseases more often (6,21) and use NSAIDs more frequently (8,23,25). Svanes et al found that increasing mean age of patients may conceal better results in the management of PPU patients, and hence mortality figures do not increase when adjusting to age groups (26). In our study the mean age of patients (45.5 years) and the proportion of female patients (17.4%) were considerably lower, and overall mortality (5.6%) was also lower compared with the above studies. We believe that such low mortality rate in our series can be largely explained by the low mean age of patients.

There has been much discussion about the choice of surgical procedure in case of emergency operation. In our study 245 non-definitive operations with a mortality of 7.3% and 141 definitive operations with a mortality of 1.4% were performed. However, comparison of the patients of the two groups (**Table 4**) revealed that higher mortality rates for non-definitive operations resulted from the effect of the surgeon's decision on the choice of operation. Patients with probable risk factors served as more eligible candidates for non-definitive procedure, since it was thought to be safer. Our results of multifactorial analysis revealed that operative method had no independent effect on mortality. Numerous data give evidence of better long-term results after definitive operation: recurrence rate is lower and the patient's self-estimation of his/her condition is higher (27-29). Several studies have confirmed that there is no reason to fear for higher mortality in emergency situation when definitive procedures are applied (6,9,30,31); in this respect non-definitive operations appear to have no advantages even in high risk patients (10,32,33). Our results demonstrate agreement with the above investigations. However, definitive surgery for PPU has shown fast decline, which is mainly supported by new ideas as well as the knowledge of the pathogenesis of ulcer disease and of the role of *Helicobacter pylori* in it (12-14). At the same time, the importance of *H. pylori* infection itself in perforated peptic ulcer is not clear. In contrast with opinions in favor of the crucial role of *H. pylori* (34-38) several researches have opposed to this idea (39-42), maintaining that PPU represents even a specific subgroup of peptic ulcer disease (43).

Different studies have described about ten risk factors predicting outcome and morbidity in PPU patients, the most frequent being old age, concomitant diseases, treatment delay and shock on admission (7-9,19,22,31,44), but also gastric ulcer location (9,20,26), hospitalization at the time of perforation (9), large diameter of perforation (45), use of steroids (9), leukocyte rate less than 9.500/mm^3 (44) and reperforation (8). Univariate analysis of our data revealed that high age (≥65 years), concomitant diseases, treatment delay ≥12 hours, female sex and non-definitive operation are risk factors for mortality, whereas location of ulcer, shock on admission and previous ulcer history had no influence on mortality. However, only high age and concomitant diseases remained significant in multivariate analysis. Apart from non-definitive surgery discussed above, neither treatment delay or female sex had independent effect on mortality in multifactorial analysis. The influence of the

female sex on outcome appeared to be a biased effect as well, as the mean age of women was significantly higher than that of males.

Wakayama et al concluded that old age alone contributed to lethal outcome (44), while others found that old age alone (without concurrent factors) did not predict outcome (7,8). Our results are in accord with the studies where old age *per se* was found to predict outcome.

In conclusion, patients' high age and presence of concomitant diseases predicted outcome after surgical procedure performed for PPU. The result did not depend on the fact whether definitive or non-definitive operations were applied.

REFERENCES

1 **Paimela H, Tuompo PK, Perikylä T, Saario I, Höckerstedt K, Kivilaakso E:** Peptic ulcer surgery during the H_2-receptor antagonist era: a population-based epidemiological study of ulcer surgery in Helsinki from 1972-1987. Br J Surg 1991; 78:28-31.

2 **Mäkelä J, Laitinen S, Kairaluoma MI:** Complications of peptic ulcer disease before and after the introduction of H_2-receptor antagonists. Hepatogastroenterology 1992; 39:144.

3 **Gustavsson S, Kelly KA, Melton LJ, Zinsmeister AR:** Trends in peptic ulcer surgery. Gastroenterology 1988; 94:688-694.

4 **Hermansson M, Stael von Holstein C, Zilling T:** Peptic ulcer perforation before and after the introduction of H_2-receptor blockers and proton pump inhibitors. Scand J Gastroenter 1997; 32:523-529.

5 **Svanes C, Salvesen H, Stangeland L, Svanes K, Soreide O:** Perforated peptic ulcer over 56 years. Time trends in patients and disease characteristics. Gut 1993; 34:1666-1671.

6 **Gumshefski L, Flanchaum L, Brolin RE, Frankel A:** Changing patterns in perforated peptic ulcer disease. Am Surg 1990; 56:270-274.

7 **Boey J, Wong J, Ong GB:** A prospective study of operative risk factors in perforated duodenal ulcers. Ann Surg 1982; 195:265-269.

8 **Evans JP, Smith R:** Predicting poor outcome in perforated peptic ulcer disease. Aust NZ J Surg 1997; 67:792-795.

9 **Hamby LS, Zweng TN, Strodel WE:** Perforated gastric and duodenal ulcer: An analysis of prognostic factors. Am Surg 1993; 59:319-324.

10 **Schein M, Gecelter G, Freinkel Z:** APACHE II in emergency operations for perforated ulcers. Am J Surg 1990; 159:309-313.

11 **De Boer WA:** Perforated duodenal ulcer. N Engl J Med 1997; 337:1013.

12 **Rober HD, Imbof M, Goretzki PE, Ohmann C:** Ulcer surgery '96 - choice of methods in an emergency. Chirurg 1996; 67:20-25.

13 **Al-Assi MT, Graham DY:** Peptic ulcer disease, *Helicobacter pylori*, and the surgeon: Changing of the guard. Curr Opin Gen Surg 1994:120-124.

14 **Kozol RA:** Surgery for peptic ulcer in the *Helicobacter pylori* era. Arch Surg 1995; 130:1040-1041.

15 **Stabile BE, Passaro E Jr:** Duodenal ulcer: A disease in evolution. Curr Probl Surg 1984; 21:6-17.

16 **Negre J:** Seasonal periodicity of peptic ulcer: a myth. Lancet 1985; i:1504-1505. (Letter)

17 **Christensen A, Bousfield R, Christiansen J:** Incidence of perforated and bleeding peptic ulcers before and after the introduction of H_2-receptor antagonists. Ann Surg 1988; 207:4-6.

18 **De Bakey M:** Acute perforated gastroduodenal ulceration. Surgery 1940; 8:852.

19 **Mattingly SS, Ram MD, Griffin WO Jr:** Factors influencing morbidity and mortality in perforated duodenal ulcer. Am Surg 1980; 46:61-66.

20 **Horgwitz J, Kukora JS, Ritchie WP:** All perforated ulcers are not alike. Ann Surg 1989; 209:693-696.

21 **Bulut O, Rasmussen C, Fischer A:** Acute surgical treatment of complicated peptic ulcers with special reference to the elderly. World J Surg 1996; 20:574-577.

22 **Irvin TT:** Mortality and perforated peptic ulcer: A case for risk stratification in elderly patients. Br J Surg 1989; 76:215-218.

23 **Cocks JR:** Perforated peptic ulcer - the changing scene. Dig Dis 1992; 10:10-16.

24 **Hudson N:** Excess long-term mortality in patients with ulcer complications. Lancet 1997; 349:968-969.

25 **Suter M:** Surgical treatment of perforated peptic ulcer. Is there a need for a change? Br J Surg 1995; 82:1140-1141.

26 **Svanes C, Salvesen H, Espehaug B, Soreide O, Svanes K:** A multifactorial analysis of factors related to lethality after treatment of perforated gastroduodenal ulcer 1935-85. Ann Surg 1989; 209:418-423.

27 **Jordan PH, Thornby J:** Perforated pyloroduodenal ulcers. Long-term results with omental patch closure and parietal cell vagotomy. Ann Surg 1995; 221:479-488.

28 **Robles R, Parrilla P, Lujan JA, Torralba JA, Cifuentes J, Liron R, Pinero A:** Short note: Long-term follow-up of bilateral truncal vagotomy and pyloroplasty for perforated duodenal ulcer. Br J Surg 1995; 82:665.

29 **Peetsalu A, Vardja T, Peetsalu M, Väli T:** Long-term results of surgical treatment of bleeding duodenal ulcers. Br J Surg 1994; 81:57.

30 **Welch CE, Rodkey GV, Gryska PR:** A thousand operations for ulcer disease. Ann Surg 1986; 204:454-467.

31 **McIntosh JH, Berman K, Holliday FM, Byth K, Chapman R, Piper DW:** Some factors associated with mortality in perforated peptic ulcer: A case control study. J Gastroenterol Hepatol 1996; 11:82-87.

32 **Bennett KG, Cannon JP, Organ CH:** Is duodenal ulcer perforation best treated with vagotomy and pyloroplasty? Am J Surg 1985; 150:743-747.

33 **DiQuinzio C, Phang PT:** Surgical management of perforated benign gastric ulcer in high-risk patients. Can J Surg 1992; 35:94-97.

34 **Sebastian M, Chandran VP, Elashaal YI, Sim AJ:** *Helicobacter pylori* infection in perforated ulcer disease. Br J Surg 1996; 82:360-362.

35 **Tokunaga J, Hata K, Ryo J, Kitaoka A, Tokuka A, Ohsumi K:** Density of *Helicobacter pylori* infection in patients with peptic ulcer perforation. J Am Coll Surg 1998; 186:659-663.

36 **Ng EK, Chung SC, Sung JJ, Lam YH, Lee DW, Lau JY, Ling TK, Lau WY, Li AK:** High prevalence of *Helicobacter pylori* infection in duodenal ulcer perforations not caused by non-steroidal anti-inflammatory drugs. Br J Surg 1997; 84:1029-1030.

37 **Corvera CU, Kirkwood SK:** Recent advances: General surgery. BMJ 1997; 315:586-589.

38 **Mihmanli M, Igor A, Kabukcuoglu F, Turkay B, Cikla B, Baykan A:** The effect of *H. pylori* in perforation of duodenal ulcer. Hepatogastroenterology 1998; 45:1610-1612.

39 **Reinbach DH, Cruckshank G, McColl KEL:** Acute perforated duodenal ulcer is not associated with *Helicobacter pylori* infection. GUT 1993; 34:1334-1337.

40 **Matsukura N, Onda M, Tokunaga A, Kato S, Yoshiyuki T, Hasegawa H, Yamashita K, Tomtitchong P, Hayashi A:** Role of *Helicobacter pylori* infection in perforation of peptic ulcer: an age- and gender-matched case-control study. J Clin Gastroenterol 1997; 25(Suppl 1): S235-S239.

41 **Chowdhary SK, Bhasin DK, Panigrahi D, Malik AK, Kataria RN, Behra A, Roy P, Singh K:** *Helicobacter pylori* infection in patients with perforated duodenal ulcer. Tropical Gastroenterology 1998; 19:19-21.

42 **Beales ILP:** *Helicobacter pylori* and peptic ulcer surgery. Br J Surg 1998; 85:571.

43 **Beales ILP:** Claim for major advance in treatment of perforated peptic ulcer seems premature. BMJ 1998; 316:860-861.

44 **Wakayama T, Ishizaki Y, Mitsusada M, Takahashi S, Wada T, Fukushima Y, Hattori H, Okuyama T, Funatsu H:** Risk factors influencing the short-term results of gastroduodenal perforation. Surg Today 1994; 24:681-687.

45 **Paunescu V, Spircu T:** Gastroduodenal: Risk factors in the outcome of a perforated gastric ulcer: a multifactorial analysis. Br J Surg 1995; 82:51.

III

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ORIGINAL ARTICLE

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Perforated peptic ulcer: is there a difference between Eastern Europe and Germany?

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Abstract *Background and aims*: Ulcer surgery and the epidemiology of peptic ulcer perforation have changed considerably in recent decades. *Patients/methods*: Within two prospective studies, 170 perforated peptic ulcer patients from 12 Eastern European centres and 37 patients from 11 German centres were analysed. *Results*: The median age of patients was 43 years in the Copernicus study and 49 years in the MEDWIS study (P=n.s.), being higher for MEDWIS female patients (73 vs 53 years, respectively; $P<0.05$). Female patients made up 17% (29/170) of the Copernicus study and 35% (40/170) of the MEDWIS study ($P<0.05$). Twenty-three per cent (40/170) of patients in the Copernicus study and 54% (20/37) in the MEDWIS study had gastric ulcer perforation ($P<0.001$). The proportion of definitive operations was higher in Eastern Europe (41.1%; 67/163) than it was in Germany (16.1%; 5/31) ($P<0.01$). German patients experienced more general complications than Eastern European patients (35 vs 12%, respectively; $P<0.01$) and a higher mortality [13% (5/37) vs 2% (4/170), respectively; $P<0.01$]. Delayed admission ≥12 h and age ≥60 years remained predictors for complications in multivariate logistic regression analysis. *Conclusion*: The proportion of both women and gastric ulcers was higher among German patients, while Eastern European patients underwent more definitive operations. German patients experienced more general complications and a higher mortality. Complications were related to high age and delayed admission.

Key words Perforated peptic ulcer · Operative treatment · Complications · Risk factors

Introduction

Substantial changes in the conservative therapy of ulcer disease have significantly reduced elective peptic ulcer surgery since the 1980s. However, no decrease has been noted in emergency ulcer surgery [1, 2,3], including that for perforated peptic ulcer (PPU). The epidemiology of PPU in developed countries has changed considerably in recent decades. Median age at the time of perforation has increased by 10–20 years (about 60–70 years in the 1990s) [4,5]. Being previously a disease predominantly affecting men [male:female (M:F) ratio about 10:1], PPU is now increasingly affecting elderly women (M:F ratio 1.5:1) [2, 3,4]. The use of non-steroidal anti-inflammatory drugs (NSAIDs) among PPU patients has increased [6,7]. The rate of complications and mortality have not declined during recent decades. Mortality is reported to vary between 5 and 31% [2, 4, 5, 7, 8, 9,10]. Higher age, delayed treatment, concomitant diseases, presence of shock on admission and location of the ulcer in the gastric corpus have been cited as the main risk factors for complications and mortality [7, 9, 11, 12, 13,14]. During

recent decades, non-definitive operative methods, mainly ulcer suturation (also performed by laparoscopic methods), are increasingly being advocated for optimal surgical treatment of PPU [6, 15, 16, 17,18]. The aim of our study was to compare PPU patients' characteristics, treatment policies, complications and the outcome of an Eastern European multicentre study (Copernicus) with the respective data of a German study (MEDWIS).

Patients and methods

Sixteen centres from nine countries participated in an Eastern European prospective multinational study of acute abdominal pain (Copernicus European Concerted Action 555) [19]. Ten centres from Germany and one from Austria participated in a similar study of acute abdominal pain (MEDWIS A 70) [20]). In both studies, all patients who experienced acute abdominal pain within 1 week before hospital admission were included. A structured, standardised medical history was drawn up for all patients and a physical examination was performed according to international standards. These clinical findings together with the data of laboratory investigations, ultrasound, X-ray, diagnosis, operative procedure and outcome were documented prospectively using a computer program. A software package with multilingual program versions was developed for documentation. The same standardised definitions were used for all parameters in both studies. Data collected at the centres were transmitted anonymously to the central study secretariat in Düsseldorf and were examined with respect to completeness and quality. Physicians participating in the study were contacted in cases of missing or unclear data. Patient recruitment took place between 1 January and 31 October 1997 for the Copernicus study; 4020 patients from 12 centres were included, among whom 170 (4.2%) had a final diagnosis of PPU. The MEDWIS study, which included a total of 2280 patients, of whom a final diagnosis of PPU was confirmed in 37 (1.6%), was conducted between October 1994 and March 1996. All patients with a final diagnosis of PPU were analysed in this particular report. Data for PPU patients' characteristics, performed operations, outcome and mortality were compared for the two studies. Ulcerogenic drugs included NSAIDs and glycocorticoids. Non-definitive operations included ulcer excision or suturation, while definitive operations included vagotomy and gastric resection. The risk factors for complications in PPU patients were evaluated by pooling the data of the two studies. In the comparison of characteristics for each variable, patients with missing data were excluded. Medians with percentiles of 25–75 were calculated for age. Absolute numbers were compared by means of the chi-square test with continuity correction. The Mann–Whitney test was used for continuous variables. Multivariate analysis with stepwise logistic regression (BMDP LR) involved variables that proved to be significant in univariate analysis using the default values of the program (enter limit: 0.1, remove limit: 0.15).

Results

Among the 170 Copernicus patients with PPU, male patients outnumbered females (M:F) by a ratio of 141:29. Among the 37 MEDWIS patients with PPU, the M:F ratio was only 24:13 (Table 1) ($P<0.05$). Median age was 43.0 (33.0–55.3) years for the Copernicus patients and 49.0 (32.0–73.0) years for the MEDWIS patients (P=n.s.). However, for female patients, it was 53.0 (40.5–71.5) years in the Copernicus study and 73.0 (55.0–82.0) years in the MEDWIS study ($P<0.05$). There was a significant difference in ulcer location between the studies, gastric ulcer perforations accounting for 23.5% (40/170) in the Copernicus study and 54.1% (20/37) in the MEDWIS study ($P<0.001$). Comparison of the two studies revealed no significant differences in terms of delay on admission, shock on admission, previous dyspep-

Table 1 Characteristics of patients with perforated peptic ulcer in the Copernicus (n=170) and MEDWIS (n=37) studies. *n.s.* not significant

Variable		Copernicus		MEDWIS		P value
		n	%	n	%	
Sex	Male	141	82.9	24	64.9	<0.05
	Female	29	17.1	13	35.1	
Ulcer location	Duodenal	130	76.5	17	46.9	<0.001
	Gastric	40	23.5	20	54.1	
Delay on admission[a] (h)	<12	125	75.8	24	64.9	
	12–24	26	15.8	8	21.6	n.s.
	>24–48	5	3.0	0	0.0	
	>48	9	5.5	5	13.5	
Shock on admission[a]	Yes	20	12.3	9	24.3	n.s.
	No	143	87.7	28	75.7	
Previous dyspepsia[a]	Yes	97	60.6	20	58.8	n.s.
	No	63	39.4	14	41.2	
Previously diagnosed ulcer[a]	Yes	63	41.7	11	34.4	n.s.
	No	88	58.3	21	65.6	
Ulcerogenic drugs[a]	Yes	13	8.1	6	18.8	n.s.
	No	148	91.9	26	81.3	

[a] Patients with missing data excluded

Table 2 Distribution of operations in patients with perforated peptic ulcer (PPU) (patients with missing data excluded)

	Copernicus		MEDWIS		*P* value
	n	%	*n*	%	
Nondefinitive operations	96	58.9	26	83.9	
Oversewing of ulcer	65		10		
Ulcer excision	29		16		
Laparoscopic management	2		0		
Definitive operations	67	41.1	5	16.1	<0.01
Billroth I resection	6		3		
Billroth II resection	6		2		
TV[a]+ulcer excision	51				
SPV[b]+ulcer excision	3				
SPV[b]+ulcer suturation	1				
Total	163	100	31	100	–

[a] Truncal vagotomy.
[b] Selective proximal vagotomy.

Table 3 Complications in 31 Copernicus patients and 10 MEDWIS patients with perforated peptic ulcer (*PPU*). *n.s.* not significant

Complication	Copernicus[a] (*N*=169 N)		MEDWIS (*N*=37)		*P* value
	n	%	*n*	%	
Intraabdominal (peritonitis/abscessus)	11	6.5	2	5.4	n.s
Wound infection	9	5.3	2	5.4	n.s.
General complications	21	12.4	13	35.1	<0.01
Pneumonia	7		5		
Pulmonary failure	3		2		
Renal failure	1		1		
Hepatic failure	1		–		
Other	9		5		
Total (overall)	31	18.3	10	27.0	n.s.

[a] One patient with missing data excluded.

Table 4 Results of evaluation of risk factors for complications in perforated peptic ulcer (*PPU*) patients in univariate analysis

Variable		Complication(s) (*N*=41)		No complications (*N*=165)		*P* value
		n	%	*n*	%	
Sex	Male	28	68.3	136	82.4	<0.05
	Female	13	31.7	29	17.6	
Age (years)	<60	20	48.8	139	84.2	<0.001
	≥60	21	51.8	26	15.8	
Ulcer location	Duodenal	25	61.0	121	73.3	n.s.
	Gastric	16	39.0	44	26.7	
Operationa	Definitive	11	27.5	61	39.9	n.s.
	Non-definitive	29	72.5	92	60.1	
Delay on admissiona (h)	<12	21	53.8	127	78.4	<0.01
	≥12	18	46.2	35	21.6	
Shock on admissiona	Yes	6	15.8	23	14.3	n.s.
	No	32	84.2	138	85.7	
Previously diagnosed ulcera	Yes	16	44.4	58	39.7	n.s.
	No	20	55.6	88	60.3	
Ulcerogenic drugsa	Yes	5	13.2	14	9.1	n.s.
	No	33	86.8	140	90.9	
White blood cell counta	<9000	15	42.9	53	34.6	n.s.
	≥9000	20	57.1	100	65.4	
Study	Copernicus	31	75.6	138	83.6	n.s.
	MEDWIS	10	24.4	27	16.4	

[a] Patients with missing data excluded.

Table 5 Results of multivariate analysis of risk factors for complications in perforated peptic ulcer (PPU) patients

Variable	*P*–value β	Coefficient	Coefficient/SE exp(β)	Odds ratio
Age ≥60 years	0.001	1.512	3.83	4.54
Delay on admission ≥12 h	0.012	1.006	2.56	2.73
Constant	–	–2.220	–7.81	0.109

sia, previously diagnosed ulcer or ulcerogenic drug usage.

The distribution of operations performed on PPU patients is shown in Table 2. In the MEDWIS study only five (16.1%) patients were operated on using a definitive method, while in the Copernicus study 67 (41.1%) patients underwent a definitive operation ($P<0.01$). At the same time, the proportion of definitive surgery varied from 0 to 91% at different centres of the Copernicus study. In the MEDWIS study, no vagotomies were performed for PPU.

There were no significant differences in the overall complication rate; however, 35.1% (13/37) of MEDWIS patients experienced general complications compared with 12.4% (21/169) of Copernicus patients ($P<0.01$) (Table 3).

A total of nine patients died during the course of the study: 2.4% (4/170) from the Copernicus study and 13.5% (5/37) from the MEDWIS study ($P<0.01$). Among these nine patients, there were four males and five females with a median age of 80 (65–93) years, in whom four gastric and five duodenal ulcer perforations had been diagnosed.

Results of the comparison of 41 patients with complications and 165 patients without complications in univariate analysis for risk factors for complications are given in Table 4. Three variables – age ≥60 years ($P<0.001$), delay on admission ≥12 h ($P<0.01$) and female sex ($P<0.05$) – proved to be significant predictors for complications. On the inclusion of these three variables in multivariate analysis with stepwise logistic regression, only age ≥60 years and delay on admission ≥12 h remained significant predictors for complications in PPU patients (Table 5).

Discussion

Within two multicentre prospective studies, conducted at 12 centres in Eastern Europe and 11 centres in Germany and Austria, data were collected according to standardised protocols, which allowed the comparison of research results. Of all studied patients with acute abdominal pain, 4.2% in Eastern Europe and 1.6% in Germany had a final diagnosis of PPU, which is comparable with the results of several previously reported series [21,22,23].

The main distinctive characteristics of PPU patients in the two studies were sex and ulcer localisation. Among German and Eastern European patients, the proportion of females was 35.1 vs 17.1% and the proportion of gastric ulcer perforations was 54.1 vs 23.5%, respectively. In the German study, the proportion of females is closer to the figures reported for the Western countries in recent decades at 40–60% [2, 4, 8,10], while the respective proportion for Eastern Europe is clearly divergent. The higher proportion of gastric ulcers in the German study is in agreement with data indicating an increasing trend in Western patients [2,4].

Median age did not reveal any significant difference, being 43 years for Copernicus patients and 49 years for MEDWIS patients. Both figures are lower than the previously reported median age of 62–66 years [4,10]. However, the higher median age of German female patients compared with that of their Eastern European counterparts (73 vs 53 years) can be explained by the above-described trends in the Western countries.

The other variables, i.e. delay on admission, shock on admission and previously diagnosed ulcer disease, did not reveal differences between Eastern European and German patients. There were relatively few users of ulcerogenic drugs in either study (8.1 and 18.8%, respectively) compared with the reported proportion of 40–70% [5, 24, 25,26].

The distribution of different operations in the two studies was quite different, with definitive operations accounting for 16.1% in the German study and 41.1% in the Eastern European study. At different Eastern European hospitals, the proportion of definitive surgery varied in the range of 0–91%, while five centres only used non-definitive operations. At the same time, in the German study, only gastric resections were used among the five definitive operations, whereas in the Eastern European study, besides 12 gastric resections, 55 operations combined with vagotomy were also performed. The high proportion of definitive operations is a characteristic feature of Eastern Europe. By contrast, according to the data of recent reports, the number of definitive procedures is diminishing rapidly in the Western countries [10, 16, 27, 28,29].

Overall complication rates – 18.3% in the Copernicus study and 27.0% in the MEDWIS study – did not reveal significant differences; however, the proportion of patients with general complications was 12.4 and 35.1% ($P<0.01$), respectively. Like the other variables, the overall complication rate in German patients is closer to 30–57% reported in most Western studies [12, 30,31], while the figure for Eastern European patients is comparable with that reported by Boey et al. [32], i.e. 14%.

Higher figures for general complications in German patients can be attributed to the different structure of the patient sample in either study. The higher proportion of females with higher median age may play some role in the development of general complications, as far as a trend of increase in general complications with the ageing of patients has been observed in the Western countries [4].

Differences in mortality – 13.5% for the MEDWIS patients and 2.4% for the Copernicus patients – can also be explained by the different structure of the two patient samples. All patients who died were at least 65 years old.

The mortality rate of 13.5% in the MEDWIS study was comparable with the rate of 6–31% reported for the Western countries in the 1990s [5, 10, 12], while the rate of 2.4% for Copernicus patients was significantly lower.

In univariate analysis, age ≥60 years, delayed treatment ≥12 h and female sex were significant predictors in the development of complications. In multivariate analysis, however, only high age and delayed treatment remained independent predictors for complications. These two factors have also been pointed out in a number of other studies [11, 14, 30, 31, 32,33]. Complications were not related to either ulcer location or to the applied operation method.

Conclusion

In the German study, the proportion of both women among PPU patients and gastric ulcer perforations were higher than that of the respective characteristics for Eastern European patients. In addition, German female patients had a higher median age. The number of general complications and the mortality rate were higher in the German study. Non-definitive operations prevailed in the treatment of PPU in German patients, while the percentage of definitive operations was significantly higher for their Eastern European counterparts. The main complication related risk factors – delayed admission ≥12 h and age ≥60 years – were comparable with the data of most reports.

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References

1. Paimela H, Tuompo PK, Peräkylät T, Saario I, Höckerstedt K, Kivilaakso E (1991) Peptic ulcer surgery during the H_2-receptor antagonist era: a population based epidemiological study of ulcer surgery in Helsinki from 1972 to 1987. Br J Surg 78:28–31
2. Aeberhard P, Lichtenhahn P, Villiger P (1990) Current status of therapy for gastroduodenal ulcer. Schweiz Med Wochensch 120:467475
3. Bloom BS, Fendrick AM, Ramsey SD (1990) Changes in peptic ulcer and gastritis/duodenitis in Great Britain, 1970–85. J Clin Gastroenterol 12:100–108
4. Svanes C, Salvesen H, Stangeland L, Svanes K, Soreide O (1993) Perforated peptic ulcer over 56 years. Time trends in patients and disease characteristics. Gut 34:1666–1671
5. Bulut OB, Rasmussen C, Fischer A (1996) Acute surgical treatment of complicated peptic ulcers with special reference to elderly. W J Surg 20:574–577
6. Cocks JR (1992) Perforated peptic ulcer – the changing scene. Dig Dis 10:10–16
7. Suter M (1995) Surgical treatment of perforated peptic ulcer. Is there a need for a change? Br J Surg 82:1140–1141
8. Mäkelä J, Laitinen S, Kairaluoma MI (1992) Complications of peptic ulcer disease before and after the introduction of H_2-receptor antagonists. Hepato-Gastroenterology 9:144–148

9. Khorsovani C, Kohen M, Guiberteau B, Le Neel JC (1994) Perforation of duodenopyloric ulcers. Prognostic factors and therapeutic choices. Retrospective study of 140 patients. Ann Chir 48:345–349
10. Hermansson M, Stael von Holstein C, Zilling T (1997) Peptic ulcer perforation before and after the introduction of H_2-receptor blockers and proton pump inhibitors. Scand J Gastroenterol 32:523–529
11. Svanes C, Espehaug B, Salvesen H, Søreide O, Svanes K (1989) A multifactorial analysis of factors related to lethality following treatment of perforated gastroduodenal ulcer 1935–85. Ann J Surg 209:418–423
12. Evans JP, Smith R (1997) Predicting poor outcome in perforated peptic ulcer disease. Aust NZ J Surg 67:792–795
13. McIntosh JH, Berman K, Holliday FM, Byth K, Chapman R, Piper DW (1996) Some factors associated with mortality in perforated peptic ulcer: a case control study. J Gastroenterol Hepatol 11:82–87
14. Hamby LS, Zweng TN, Strodel WE (1993) Perforated gastric and duodenal ulcer: analysis of prognostic factors. Am Surg 59:319–324
15. De Boer WA (1997) Perforated duodenal ulcer. New Engl J Med 337:1013
16. Roher HD, Imhof M, Goretzki PE, Ohmann C (1996) Ulcer surgery '96 – choice of methods in an emergency. Chirurg 67:20–25
17. Druart ML, Van Hee R, Etienne J, Cadiere GB, Gigot JF, Legrand M, Limbosch JM, Navez B, Tugilimana M, Van Vyve E, Vereecken L, Wibin E, Yvergneaux JP (1997) Laparoscopic repair of perforated duodenal ulcer. A prospective multicenter clinical trial. Surg Endosc 11:1017–1020
18. Lau WY, Leung KL, Zhu XL, Lam YH, Chung SC, Li AK (1995) Laparoscopic repair of perforated peptic ulcer. Br J Surg 82:814–816
19. Ohmann C, Eich H-P, Sippel H and Copernicus study group (1998) A data dictionary approach to multilingual documentation and decision support for the diagnosis of acute abdominal pain (Copernicus 555, An European Concerted Action). In: Cesnik B, McCray AT, Scherrer JR (eds). Proceedings of the 9th World Conference on Medical Informatics (MEDINFO 98). IOS Press, Amsterdam, pp. 462–466
20. Kraemer M, Kremer K, Leppert R, Yang Q, Ohmann C, Fuchs KH (1999) Perforating appendicitis: is it a separate disease? Eur J Surg 165:473–480
21. Ikonen JK, Rokkanen PU, Grönroos P, Kataja JM, Nykänen P, de Dombal FT, Softley A (1983) Presentation and diagnosis of acute abdominal pain in Finland: a computer aided study. Ann Chir Gynaecol 72:332–336
22. De Dombal FT (1988) The OMGE acute abdominal pain survey. Progress Report 1986. Scand J Gastroenterol 23 [Suppl 144]:35–42
23. Ohmann C, Kraemer M, Jäger S, Sitter H, Stadelmayer B, Vietmeier P, Wickers J, Latzke L, Koch B, Thon K (1992) Akuter Bauchschmerz – standardisierte Befundung als Diagnoseunterstützung. Chirurg 63:113–123
24. Bliss DW, Stabile BE (1992) The impact of ulcerogenic drugs on surgery for the treatment of peptic ulcer disease. Arch Surg 126:609–612
25. Lanas A, Serrano P, Bajador E, Esteva F, Benito R, Sainz R (1997) Evidence of aspirin use in both upper and lower gastrointestinal perforation. Gastroenterology 112:683–689
26. Svanes C, Overbo K, Soreide O (1996) Ulcer bleeding and perforation: nonsteroidal anti-inflammatory drugs or *Helicobacter pylori*. Scand J Gastroenterol 220:128–131
27. Al-Assi MT, Graham DY (1994) Peptic ulcer disease, *Helicobacter pylori*, and the surgeon: changing of the guard. Curr Opin Gen Surg:120–124
28. Kozol RA (1995) Surgery for peptic ulcer in the *Helicobacter pylori* era. Arch Surg 130:1040–1041
29. Blomgren LG (1997) Perforated peptic ulcer: long-term results after simple closure in the elderly. W J Surg 21:412–415
30. Gunshefski L, Flancbaum L, Brolin RE, Frankel A (1990) Changing patterns in perforated peptic ulcer disease. Am Surg 56:270–274
31. Wakayama T, Ishizaki Y, Mitsusada M (1994) Risk factors influencing the short term results of gastroduodenal perforation. Surg Today 24:681–687
32. Boey J, Wong J, Ong GB (1982) A prospective study of operative risk factors in perforated duodenal ulcer. Ann Surg 195:265–269
33. Mattingly SS, Ram MD, Griffin WO Jr. (1980) Factors influencing morbidity and mortality in perforated ulcer. Am Surg 46:61–66

IV

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Diversity of *Helicobacter pylori* genotypes among Estonian and Russian patients with perforated peptic ulcer, living in Southern Estonia

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Abstract

To compare the genomic variation of *Helicobacter pylori* in samples obtained from patients with perforated peptic ulcer, living in the same area of Estonia but belonging to different nationalities, 50 non-consecutive patients (32 Estonians and 18 Russians) admitted in the Tartu University Hospital in 1997–1999 were studied. Gastric samples of antral mucosa were obtained during operation and analysed histologically and with PCR for detection of different genotypes of *H. pylori* (*cagA* and *vacA* s and m subtypes). Among the 50 perforated peptic ulcer patients with histologically proven *H. pylori* colonisation no sample of gastric mucosa showed the s1b subtype of the *vacA* gene. The perforated peptic ulcer patients were mainly infected with *cagA* (82%) and s1 (98%) genotypes of *H. pylori*. The distribution of s1a/m1, s1a/m2 and s2/m2 subtypes of *vacA* genes was statistically different in Estonian and Russian patients ($P < 0.05$). In conclusion differences in the distribution of *vacA* s and m subtypes of *H. pylori* were revealed between Estonian and Russian patients with perforated peptic ulcer from Southern Estonia.

Keywords: PCR; Ethnic tropism; *cagA*; *vacA*; *Helicobacter pylori*

1. Introduction

Helicobacter pylori plays an important role in gastritis, peptic ulcer disease and gastric malignancy [1]. To date, several products of *H. pylori* genes (*vacA* and its subtypes, *cagA*, *ice*), acting as disease-associated pathogenic factors in infected persons, have been identified [2–4]. A key feature of *H. pylori* is the enormous genomic micro-diversity of its strains distributed over the globe, even many individuals appear to be infected by a unique strain.

At the same time, different countries seem to be characterised by a particular geographic pattern of different *H. pylori* genotypes [5,6]. In Western populations, gastric atrophy, duodenal ulceration, intestinal metaplasia, gastric carcinoma are more common among patients infected with *cagA* positive than among patients infected with *cagA* negative strains [4,5]. Yet, in China and Japan the association between *cagA* positivity and virulence of *H. pylori* strains was equally frequent among both diseased and control patients [5,7]. Recently, it was shown that in East Asia and in the Western countries, distinct variants of *H. pylori cagA* genes were associated with particular *vacA* subtypes [8].

However, the clinical significance of the genetic markers of *H. pylori* has not yet definitely been proved. Particularly, the incidence of peptic ulcer disease is not reflected in the frequency of different *H. pylori* lineages among Polynesians and European New Zealanders [9]. Obviously, further research is needed to compare differences in the *H. pylori* genome between particular ethnic groups with the same underlying disease, living in the same geographic region.

In Estonia, an increase in perforated peptic ulcer (PPU) has been noted since 1991–1997, the mean incidence for Southern Estonia amounting to 21/100 000 [10]. At the same time, in Western countries the incidence of PPU

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has been kept in the range less than 10/100 000 in recent decades [11]. Estonia is a state in Eastern Europe, lying on the coast of the Gulf Finland, with a population of about 1.5 million and area of 45 000 km^2. The Estonian population consists of two-thirds of native Estonians and one-third of other nationalities, mainly Russians. In the present study an attempt was made to compare the genomic variation of *H. pylori* in samples obtained from patients with PPU, living in the same area of Estonia but belonging to different nationalities.

2. Materials and methods

2.1. Patients

The study was carried out at Tartu (Southern Estonia) University Hospital from January 1, 1997 to December 31, 1999. During this period 129 patients with PPU were hospitalised. Informed consent was obtained from all patients who were questioned using a standard questionnaire. Only the patients ($n = 53$) of whom both parents could be identified as being of the same nationality were included. All patients were operated and three gastric specimens of the antral mucosa were taken intra-operatively through the perforation, or postoperatively on pan-endoscopy. Two of the specimens were analysed by histological methods and one by molecular methods.

2.2. Histological evaluation

Biopsy specimens were fixed overnight in neutral buffered formalin and embedded in paraffin. Tissue sections were stained by the modified Giemsa method for semi-quantitative assessment of *H. pylori* colonisation as described earlier [12].

2.3. Molecular evaluation

For analysis with molecular methods the specimens were placed into 500 μl of lysis buffer (200 mM Tris–HCl (pH 8.0), 25 mM EDTA, 300 mM NaCl, 1.2% sodium dodecyl sulfate) and stored at −20°C. Different genotypes (*cagA* and *vacA* s and m subtypes) of *H. pylori* were assessed. DNA was extracted from a frozen gastric biopsy specimen using the following procedure: 10 μl of the proteinase K (400 μg ml^{-1}) was added to the biopsy specimen suspended in the lysis buffer (500 μl). The mixture was incubated at 37°C for 24 h. The lysate was extracted with an equal volume of phenol–chloroform and precipitated with ethanol. A DNA pellet was collected by centrifugation, washed with 70% ethanol and finally resuspended in 35–50 μl of TE buffer (10 mM Tris–HCl (pH 8.0), 0.1 mM EDTA). For analysis of the s and m regions of *vacA* and for detection of the *cagA* gene, the primers shown in Table 1 were used.

2.4. PCR conditions and amplification

Reaction mixtures were prepared in a volume of 50 μl, containing 0.3 μM concentration of each primer; 0.2 mM concentration of deoxynucleoside triphosphates; 10× reaction buffer (200 mM Tris–HCl (pH 8.5), 500 mM KCl, 10 mM $MgCl_2$, 0.01% (w/v) gelatin); 1 mg ml^{-1} albumin (BSA); 2.5 mM $MgCl_2$; 5 U of Taq DNA polymerase (Fermentas); and ~10 ng DNA sample. The mixtures were placed in a PCR thermocycler (Biometra, Eppendorf). Thermal cycling for all primer pairs comprised 4 min of preincubation at 95°C, followed by one cycle of 1 min at 95°C, 1 min 10 s at 56°C, 30 s at 72°C; three cycles of 1 min at 95°C, 1 min 10 s at 54°C, 30 s at 72°C; 36 cycles of 1 min 95°C, 1 min 10 s at 52°C, 30 s at 72°C. PCR products were identified by electrophoresis on 2% agarose gels.

2.5. Statistical analysis

The mean values of patients' age were compared by employing the Student *t*-test. The absolute figures of prevalence were compared by using the Chi-square test with a continuity correction. Differences were considered statistically significant for *P* values less than 0.05.

Table 1
PCR primers for amplification of *vacA* sequences and *cagA* gene of *H. pylori*

Amplified region	Primer destination	Primer sequence	Product size (bp)	Source of reference
s1	VA1-F	ATGGAAATACAACAAACACAC		[2]
	VA1-R	CTGCTTGAATGCGCCAAAC	259	
s1a	SS1-F[a]	GTCAGCATCACACCGCAAC	190	
s1b	SS3-F[a]	AGCGCCATACCGCAAGAG	187	
s2	SS2-F[a]	GCTAACACGCCAAATGATCC	199	
m1	VA3-F	GGTCAAAATGCGGTCATGG		
	VA3-R	CCATTGGTACCTGTAGAAAC	290	
m2	VA4-F	GGAGCCCCAGGAAACATTG		
	VA4-R	CATAACTAGCGCCTTGCAC	352	
cagA	D008	ATAATGCTAAATTAGACAACTTGAGCGA		[13,14]
	R008	TTAGAATAATCAACAAACATCACGCCAT	297	

[a]Used in combination with primer VA1-R.

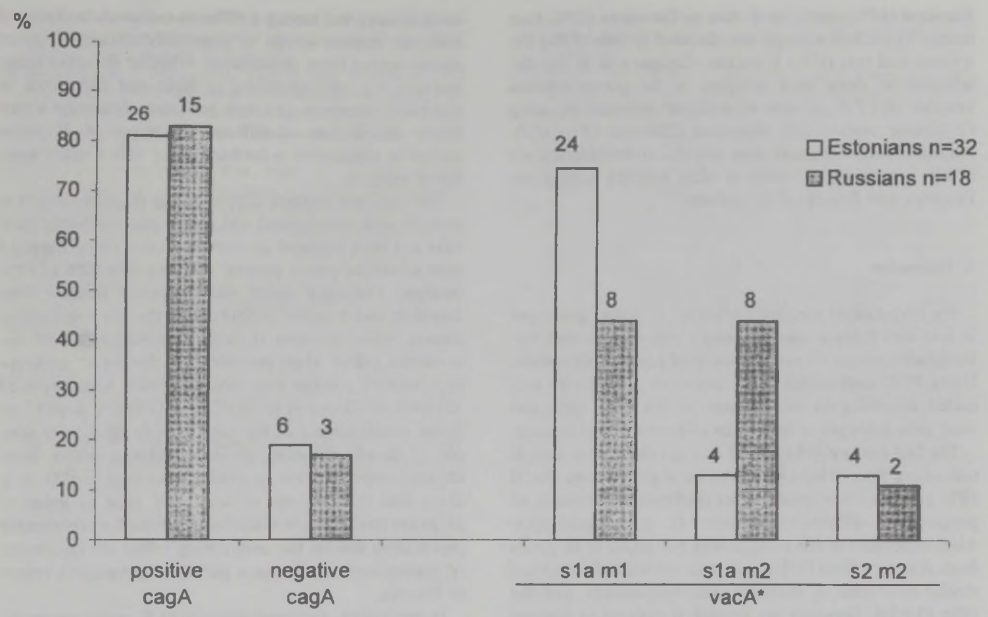

Fig. 1. *cagA* and *vacA* status of *H. pylori* in antral mucosa samples of Estonian and Russian patients with PPU (*$P = 0.037$).

3. Results

H. pylori was detected histologically in the antral mucosa of 52 out of 53 included PPU patients (98%). The degree of colonisation in the antral mucosa varied from grade 1 to grade 3 (grade 1: < 20 microbes per field; grade 2: 20–60 microbes per field; grade 3: > 60 microbes per field).

Using a PCR technique *H. pylori* was detected in the antral mucosa of 51 PPU patients (96%). In one patient with no detected *vacA* or *cagA* genotype, *H. pylori* was still proven to be present histologically.

In one Estonian male with a *cagA* positive sample, two *vacA* gene subtypes (s1a/m1 and s1a/m2) were found simultaneously and the patient was excluded from further analysis when comparing *H. pylori* subtypes in persons of different nations. The distribution of the remaining 50 *H. pylori* positive samples from 32 Estonians and 18 Russians according to the markers of *cagA* and *vacA* gene subtypes is presented in Table 2. Among *H. pylori* positive samples, 41 (82%) were *cagA* positive. The most frequent was the s1a/m1 *vacA* subtype (32 cases), while the s1b subtype was not found in our PPU patients.

Both s1 subtypes (s1a/m1 and s1a/m2) prevailed (98%) in *cagA* positive samples. Comparison of the distribution of the pattern of three *vacA* subtypes in patients with *cagA* positive and negative strains by using Chi-square test yielded statistical difference at the level $P < 0.001$ (Table 2).

No differences were observed in the distribution of *cagA* positive or negative markers between Estonian and Russian patients with PPU, as the *cagA* gene was revealed in 81% (26 of 32) and 83% (15 of 18) of cases, respectively (Fig. 1). In contrast, the distribution of *vacA* subtypes was different in the gastric samples of our Estonian and Russian patients. The s1a/m1 subtype was found in 75% (24 of 32) of Estonians but in only 44% (8 of 18) of Russians. At the same time, the s1a/m2 subtype was more frequent in

Table 2
Distribution of *H. pylori cagA* and *vacA* status in PPU patients

vacA subtype	*cagA*$^+$, $n = 41$	*cagA*$^-$, $n = 9$	Chi-square
s1a/m1	29 (71%)	3 (33%)	
s1a/m2	11 (27%)	1 (11%)	$P < 0.001$
s2/m2	1 (2%)	5 (56%)	
Total	41 (100%)	9 (100%)	

Russians (44%, eight cases) than in Estonians (13%, four cases). The s2/m2 subtype was detected in four (13%) Estonians and two (11%) Russians. Comparison of the distribution of three *vacA* subtypes in the gastric mucosa samples of PPU patients of different nationalities using Chi-square test yielded statistical difference ($P = 0.037$). No significant differences were revealed in the distribution of age, sex, smoking habits or ulcer location among our Estonian and Russian PPU patients.

4. Discussion

We investigated the distribution of *H. pylori* genotypes in Southern Estonia among patients with complicated, histologically proven *H. pylori* associated peptic ulcer disease. Using PCR method the genotypes of *H. pylori* were estimated according to the presence of the *cagA* gene and *vacA* gene subtypes in specimens of gastric antral mucosa.

The fact that we did not find any gastric mucosa sample containing the s1b subtype of the *vacA* gene among the 50 PPU patients investigated by us confirms the existence of geographical differences between *H. pylori* genotypes. Also, although the s1b subtype was not found in *H. pylori* from Asian patients [15], it has been established in several studies conducted in Portugal, The Netherlands, and the USA [3,9,16]. However, as Estonia is situated in Eastern Europe, geographic differences between *H. pylori* genotypes cannot be linked with different continents.

Furthermore, in our study the distribution of s1a/m1, s1a/m2 and s2/m2 subtypes of *vacA* genes was statistically different in Estonian and Russian patients ($P < 0.05$), both groups living in South Estonia. This confirms data about the ethnic tropism of *H. pylori*, suggested by Campbell et al. [9]. Yet these authors associated differences between the *H. pylori* strains colonising Polynesians and Europeans in New Zealand, with race-specific specialisation of *H. pylori* separate strains. However, this cannot be the reason for *H. pylori* related ethnic differences between Estonians and Russians, as far as both nationalities belong to the same race. We suppose that different predominant strains may be circulating in a particular area.

The Estonian and Russian PPU patients studied by us showed similarity in the distribution of age, sex and smoking habits as well as ulcer localisation. Evidently, these factors are not closely associated with ethnic tropism of *H. pylori*. Regrettably, we could not follow the socio-economic conditions in case of our patients and hence it is impossible to conclude if their habits (difference in food or health status etc.) have influenced the distribution of *H. pylori* subtypes. In previous epidemiological studies these conditions were strongly associated with transmission of *H. pylori* infection. In Germany, *H. pylori* infection was established in 6.1% of native German children versus 44.8% of Turkish children [17]. However, the different incidence of *H. pylori* infection in populations from the same country but having a different economic background does not explain spread of genetically distinct *H. pylori* strains among these populations. Whether the other determinants, e.g. region/country of birth and childhood, or microbial adherence and host receptors, determine a particular distribution of different genotypes of *H. pylori* should be established in further studies with a larger number of subjects.

The virulence markers differentiating *H. pylori* strains in patients with complicated and uncomplicated peptic ulcer have not been assessed up to now. In our study the *cagA* gene of the *H. pylori* genome was found in 82% of PPU patients. The *cagA* status was similar in samples from Estonian and Russian patients with the same underlying disease, while the *vacA* s1 subtype prevailed in both nationalities (98%). High prevalence of the $cagA^+$ genotype and *vacA* s1 subtype has been associated with increased virulence of *H. pylori* strains [2,3]. However, recently we found predominance of the *vacA* s1 gene in another sample of South Estonian patients suffering either from chronic active gastritis or peptic ulcer disease [18]. It is likely that the presence of *cagA* and *vacA* s1 genes of *H. pylori* strains is not exclusively specific for complicated peptic ulcer disease but may merely reflect the circulation of predominant strains in a particular geographic region of Estonia.

In conclusion, we found diversity in *H. pylori* genotypes among Estonian and Russian patients with PPU from South Estonia. This finding points to the need of further investigations for future development of novel therapeutic targets and vaccines specific for different ethnic groups.

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References

[1] Graham, D.Y. (1997) *Helicobacter pylori* infection in the pathogenesis of duodenal ulcer and gastritis cancer: a model. Gastroenterology 113, 1983–1991.

[2] Atherton, J.C., Cao, P., Peek, R.M., Tummuru, M.K.R., Blaser, M.J. and Cover, T.L. (1995) Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. J. Biol. Chem. 30, 17771.

[3] Atherton, J.C., Peek, R.M., Tham, K.T., Cover, T.L. and Blaser, M.J. (1997) Clinical and pathological importance of heterogeneity in VacA, the vacuolating cytotoxin gene of *Helicobacter pylori*. Gastroenterology 112, 92–99.

[4] Censini, S., Lange, C., Xiang, Z., Crabtree, J.E., Ghiara, P., Borodovsky, M., Rappuoli, R. and Covacci, A. (1996) *Cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. USA 93, 14648–14653.

[5] Ito, Y., Azuma, T., Ito, S., Miyaji, H., Hirai, M., Yamazaki, Y.,

Kohli, Y. and Kuriyama, M. (1997) Analysis and typing of the vacA gene from cagA-positive strains of *Helicobacter pylori* isolated in Japan. J. Clin. Microbiol. 35, 1710–1714.

[6] Rudi, J., Kolb, C., Maiwald, M., Kuck, D., Sieg, A., Galle, P.R. and Stremmel, W. (1998) Diversity of *Helicobacter pylori vacA* and *cagA* genes and relationship to VacA and CagA protein expression, cytotoxin production, and associated diseases. J. Clin. Microbiol. 36, 944–948.

[7] Pan, Z.J., van der Hulst, R.W.M., Feller, M., Xiao, S.D., Tytgat, G.N.J., Dankert, J. and van der Ende, A. (1997) Equally high prevalence of infection with cagA-positive *Helicobacter pylori* in Chinese patients with peptic ulcer disease and those with chronic gastritis associated dyspepsia. J. Clin. Microbiol. 35, 1344–1347.

[8] van Doorn, L.-J., Figueiredo, C., Sanna, R., Blaser, M.J. and Quint, W.G.V. (1999) Distinct variants of *Helicobacter pylori* cagA are associated with vacA subtypes. J. Clin. Microbiol. 37, 2306–2311.

[9] Campbell, S., Frazer, A., Holliss, B., Schmid, J. and O'Toole, P.W. (1997) Evidence for ethnic tropism of *Helicobacter pylori*. Infect. Immun. 65, 3708–3712.

[10] Sillakivi, T., Tein, A. and Peetsalu, A. (1999) Changing incidence and surgical management of perforated peptic ulcer (PPU) in Tartu county, Estonia, 1984–1997. Ann. Chir. Gyn. 88, 168.

[11] Hermansson, M., Stael von Holstein, C. and Zilling, T. (1997) Peptic ulcer perforation before and after the introduction of H2-receptor blockers and proton pump inhibitors. Scand. J. Gastroenterol. 32, 523–529.

[12] Peetsalu, A., Maaroos, H.-I., Sipponen, P. and Peetsalu, M. (1991) Long-term effect of vagotomy on gastric mucosa and *Helicobacter pylori* in duodenal ulcer patients. Scand. J. Gastroenterol. 26 (Suppl. 186), 77–83.

[13] Domingo, D., Alarcón, T., Prieto, N., Sánchez, I. and López-Brea, M. (1999) *CagA* and *vacA* status of Spanish *Helicobacter pylori* clinical isolates. J. Clin. Microbiol. 37, 2113–2114.

[14] Covacci, A. and Rappuoli, R. (1996) PCR amplifications of *H. pylori* gene sequences. In: *Helicobacter pylori*: Techniques for Clinical Diagnosis (Lee, A. and Megraud, F., Eds.), pp. 94–111. W.B. Saunders Company Ltd., London.

[15] Atherton, J.C., Karita, M., Gonzalez-Valencia, G., Peek, R.M. and Cover, T.L. (1996) Diversity in vacA mid-region sequence but not in signal sequence type among *Helicobacter pylori* strains from Japan, China, Thailand and Peru. Gut 39, A73–A74.

[16] Pan, Z.J., van der Hulst, W.M., Tytgat, G.N.J., Dankert, J. and van der Ende, A. (1999) Relation between vacA subtypes, cytotoxin activity, and disease in *Helicobacter pylori*-infected patients from the Netherlands. Am. J. Gastroenterol. 94, 1517–1521.

[17] Bode, G., Rothenbacher, D., Brenner, H. and Adler, G. (1998) *Helicobacter pylori* and abdominal symptoms: a population-based study among preschool children in Southern Germany. Paediatrics 101, 634–637.

[18] Lõivukene, K., Kolk, H., Maaroos, H.-I., Kasenõmm, P., Aro, H., Ustav, M. and Mikelsaar, M. (2000) Metronidazole and clarithromycin susceptibility and the subtypes of *vacA* of *Helicobacter pylori* isolates in Estonia. Scand. J. Infect. Dis. 32, 59–62.

V

Sillakivi T, Peetsalu M, Mikelsaar M, Peetsalu A.
An Attempt for *Helicobacter pylori* Eradication with Intravenous Clarithromycin in Perforated Peptic Ulcer Patients.
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CORRESPONDENCE

An Attempt at Helicobacter pylori *Eradication with Intravenous Clarithromycin in Perforated Peptic Ulcer Patients*

TO THE EDITOR: To date, *Helicobacter pylori* eradication is mandatory in all patients with peptic ulcer disease. Mainly 1–2 week triple or quadruple peroral regimens are advocated. However, as opposed to uncomplicated ulcer and peptic ulcer with haemorrhage, almost no data are available about the application of the above regimens and successful eradication of *H. pylori* in perforated peptic ulcer (PPU) patients.

A non-standard intravenous regimen has already shown promising results in peptic ulcer patients. Thus Friess and co-authors (1) followed eradication of *H. pylori* in proximal vagotomy patients after a perioperative prophylactic single dose of mezlocilline and metronidazole. In a 3-month follow-up, eradication of *H. pylori* was achieved in 85% of patients. In other reports, *H. pylori* eradication rates have ranged from 44% (2) to 87% (3) in peptic ulcer bleeding patients and were 93% in gastroduodenal ulcer disease patients (4) using intravenous regimens.

Although the metronidazole resistance of *H. pylori* is high in Estonia (46%), no clarithromycin resistance has been registered (5). Clarithromycin monotherapy has been demonstrated to display one of the highest monotherapy eradication rates in vivo. However, in combination with other antibiotics and acid suppressive compounds it has been shown to increase eradication rates notably (6). For example, when clarithromycin was used in combination with ranitidine bismuth citrate, eradication of *H. pylori* was achieved in 82% of cases in uncomplicated ulcer patients (7). As clarithromycin had not been introduced in Estonia before this study, one might expect no resistance of *H. pylori* to this compound. Hence we supposed that it might be advantageous to use clarithromycin treatment for eradication of *H. pylori* infection in our PPU patients immediately after operation. As a pilot study, intravenous infusion of Klacid (0.5 g b.i.d.) was used for 5 days in 6 consecutive PPU patients starting from the 1st postoperative day. No antacids were applied at the time of treatment and in case of need only cefamandol (Mandol) was used (3 g per day) for peritonitis cure in 4 patients. In all patients, 2 specimens from the gastric corpus and 2 from the antrum for histological examination of *H. pylori* were collected through the perforation with flexible forceps during operation and 6 weeks after eradication on panendoscopy. The specimens were fixed overnight in neutral buffered formalin, embedded in paraffin, and tissue sections were stained by the modified Giemsa method. *H. pylori* was determined semiquantitatively as in our earlier studies (8). The study was approved by the Tartu University Ethics Committee and informed consent was obtained from each subject.

Patient characteristics and the results of *H. pylori* eradication are presented in Table I. Only slight score differences were seen between *H. pylori* colonization of the gastric antrum and corpus mucosa before and after treatment. Eradication of *H. pylori* was achieved in not one patient 6 weeks after treatment. The reason for this could not be clarithromycin resistance, as no resistant strains (MIC ranges

Table I. The results of *Helicobacter pylori* eradication with 5-day (0.5 g b.i.d.) intravenous clarithromycin regimen in perforated peptic ulcer patients

Patient	Ulcer location	Operation	*Helicobacter pylori* density on microscopy*	
			Before treatment	6 weeks after treatment
1. 48 years male	Corpus	Ulcer suturation	Antrum no data Corpus no data	Antrum^{+} Corpus^{++}
2. 46 years male	Bulbus	Ulcer excision	Antrum^{++} Corpus^{+}	Antrum^{++} Corpus^{-}
3. 42 years male	Pylorus	Ulcer suturation	Antrum^{+++} Corpus^{++}	Antrum^{+++} Corpus^{++}
4. 61 years male	Pylorus	Truncal vagotomy + pyloroplasty	Antrum^{++} Corpus^{++}	Antrum^{++} Corpus^{+}
5. 57 years male	Pylorus	Truncal vagotomy + pyloroplasty	Antrum^{++} Corpus^{+++}	Antrum^{++} Corpus^{++}
6. 70 years female	Bulbus	Ulcer excision	Antrum^{++} Corpus^{++}	Antrum^{+++} Corpus^{++}

* *H. pylori* density.
– No *H. pylori*.
$^{+}$ Grade 1: <20 microbes per field.
$^{++}$ Grade 2: 20–60 microbes per field.
$^{+++}$ Grade 3: >60 microbes per field.

<0.016–0.75 mg/L) had been detected before the year 1999 (5). Also, it has been proved that clarithromycin is concentrated in gastric juice in comparison with plasma and its concentration in gastric juice after intravenous administration does not depend on the circumstance whether omeprazole was co-administered or not (9). Meanwhile, it has also been proved that clarithromycin activity is reduced in an acid milieu (10). Being aware that even truncal vagotomy could not be sufficiently effective for adequate acid suppression, we still used the same regimen without any proton-pump inhibitors, irrespective of whether or not the acid suppressive component (vagotomy) in a pilot study was involved in the operation. The evaluated intravenous regimen for *H. pylori* eradication was unsuccessful in the case of either option and we abandoned its application. However, although this particular monotherapy was unsuccessful, in our opinion the idea of immediate postoperative *H. pylori* eradication in complicated peptic ulcer patients seems to deserve further investigation, especially when combining several antibiotics and acid suppressive compounds.

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References

1. Friess H, Malfertheiner P, Flock F, Baczako K, Stanescu A, Büchler M. Elimination of *H. pylori* by single shot antibiotic treatment in patients undergoing proximal gastric vagotomy. Eur J Gastroenterol Hepatol 1992;4:719–25.
2. Sheu BS, Chi CH, Yang HB, Jen CM, Lin XZ. A three-day course of intravenous omeprazole plus antibiotics for *H. pylori*-positive bleeding duodenal ulcer. Hepato-Gastroenterol 1999; 46:2363–71.
3. Romero GM, Martinez DC, Grande L, Otero Fernandez MA, Vargas J, Castro FM. Intravenous eradication therapy for bleeding gastroduodenal ulcer associated with *Helicobacter pylori* infection. Rev Enferm Dig 2000;24:299–305.
4. Adamek RJ, Wegener M, Labenz J, Freitag M, Opferkuch W, Ruhl GH. Medium-term results of oral and intravenous omeprazole/amoxicillin *Helicobacter pylori* eradication therapy. Am J Gastroenterol 1994;89:39–42.
5. Lõivukene K, Kolk H, Maaroos H-I, Kasenõmm P, Aro H, Ustav M, et al. Metronidazole and clarithromycin susceptibility and the subtypes of *vacA* of *Helicobacter pylori* isolates in Estonia. Scand J Infect Dis 2000;32:59–62.
6. Markham A, McTavish D. Clarithromycin and omeprazole as *Helicobacter pylori* eradication therapy in patients with *H. pylori* associated gastric disorders. Drugs 1996;51:161–78.
7. Meyer JM, Ryu S, Pendland SL, Danziger LH. In vitro synergy testing of clarithromycin and 14-hydroxyclarithromycin with amoxicillin or bismuth subsalicylate against *Helicobacter pylori*. Antimicrob Agents Chemother 1997;41:1607–8.
8. Peetsalu M, Maaroos H-I, Peetsalu A. Completeness of vagotomy, *Helicobacter pylori* colonization and recurrent ulcer 9 and 14 years after operation in duodenal ulcer patients. Eur J Gastroenterol Hepatol 1998;10:305–11.
9. Goddard AF, Jessa MJ, Barrett DA, Shaw PN, Idstrom JP, Cederberg C. Effect of omeprazole on the distribution of metronidazole, amoxicillin, and clarithromycin in human gastric juice. Gastroenterology 1996;111:358–67.
10. Cedebrant G, Kahlmeter G, Schalen C, Kamme C. Additive effect of clarithromycin combined with 14-hydroxy clarithromycin, erythromycin, amoxycillin, metronidazole or omeprazole against *Helicobacter pylori*. J Antimicrob Chemother 1994;34: 1025–9.

VI

Andreson H, Lõivukene K,
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Association of *cagA* and *vacA* genotypes of *Helicobacter pylori*
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Association of *cagA* and *vacA* Genotypes of *Helicobacter pylori* with Gastric Diseases in Estonia

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Gastric biopsy specimens from 156 adult patients from southern Estonia suffering from chronic gastritis, peptic ulcer disease, and perforated peptic ulcer were analyzed by PCR. The *cagA* gene was evenly distributed throughout 87% of the specimens from the patients with the different gastric diseases. The presence of the *cagA* gene correlated with that of *vacA* signal sequence type s1a (99%). However, no clear differences were found in the distribution of *cagA* and *vacA* genotypes among patients in Estonia with severe perforated peptic ulcer, uncomplicated peptic ulcer, or chronic gastritis.

Helicobacter pylori colonizes approximately half of the world's human population (6). It is considered the etiological agent of chronic gastritis (CG) and peptic ulcer and their complications (3, 14, 15, 21). In Estonia, antibodies against *H. pylori* have been detected in 80% of the inhabitants (12, 13, 30). Moreover, peptic ulcer disease (PUD) (both gastric and duodenal) is widespread in Estonia (13), and the incidence of perforated peptic ulcer (PPU) in particular is very high (21 of 100,000 cases) (19).

According to the literature, virulent cytotoxin VacA- and CagA-producing strains are more common among patients with peptic ulcer and gastric cancer (2, 4). Yet in many studies, patients with a variety of clinical symptoms of gastritis, gastric ulcer, duodenal ulcer, and reflux esophagitis have been examined for similar virulence markers of *H. pylori* (26, 27). It has not been resolved whether differences in the pathogenesis of this spectrum of diseases may be related to particular virulence markers differentially expressed by strains of *H. pylori*.

The aim of the present study was to compare the distributions of well-known virulence markers (*cagA* and *vacA*) in *H. pylori* strains isolated from southern Estonian patients suffering from CG, PUD, and PPU.

This study was carried out at Tartu University Clinic (Tartu, Estonia) between 1995 and 2000. Samples were collected from 156 adult patients from southern Estonia with CG, PUD, and PPU. Initially, 105 patients were investigated from 1 May 1995 to 30 April 2000, following referral to the Tartu University outpatient clinic for upper gastrointestinal endoscopy. In addition, samples were collected from 51 patients who were operated on for PPUs during the period from 1 January 1997 to 31 December 1999.

For the detection of CG and PUD, *H. pylori* strains were isolated from biopsy samples obtained by endoscopy. Upper endoscopy was performed with an Olympus GIF 21 gastroscope. Endoscopic diagnoses of PUD were made for 69 patients (duodenal ulcer in 61 patients and gastric ulcer in 8 patients), and diagnoses of no PUD were made for 36 patients, in whom CG was diagnosed histologically according to the Sydney classification method (16).

For *H. pylori* isolation, the biopsy samples from 93 patients with CG and PUD were placed into Stuart transport medium and taken to the laboratory within 2 h. *H. pylori* was isolated on a Columbia agar base supplemented with 7% horse blood and 1% IsoVitaleX. The plates were incubated for 3 to 4 days at 37°C under microaerophilic conditions (CampyGen; Oxoid). *H. pylori* was identified by Gram staining and by oxidase, catalase, and urease reactions. The *H. pylori* suspension in brucella broth was stored at −70°C until DNA extraction. For DNA isolation from *H. pylori* cells, the suspension was incubated at 100°C for 30 min and the lysates were stored at −20°C prior to PCR analysis. For patient samples ($n = 12$) for which attempts to cultivate *H. pylori* were unsuccessful, biopsy samples stored at −20°C in lysis buffer (200 mM Tris-HCl [pH 8.0], 25 mM EDTA, 300 mM NaCl, 1.2% sodium dodecyl sulfate) were used for PCR analysis.

From the 51 PPU patients, gastric specimens of the antral mucosa were obtained for molecular analysis intraoperatively through the perforation or postoperatively with panendoscopy. These biopsy samples were placed directly into the aforementioned lysis buffer. For DNA extraction from the frozen gastric biopsy specimens, we used a previously described procedure (20).

The presence in each strain of *cagA* and *vacA* was determined by PCR using primers, reaction mixtures, and thermal cycling (20). PCR products were identified by electrophoresis on 2% agarose gels.

Statistical analysis was performed by the two-tailed χ^2 test or by Fisher's exact test. Significance was set at a P value of <0.05.

Among the 156 patients infected with *H. pylori* strains, no s1b strains were found. Multiple *H. pylori* strains were detected in 5 (3.2%) of the 156 patients studied; these included 3 patients with duodenal ulcer disease, 1 patient with CG, and 1 patient with PPU. The results from those patients were not included in the following analysis.

Among the 151 remaining investigated samples, the *cagA*

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TABLE 1. Relationships between *vacA* signal alleles and *cagA* status of 151 *H. pylori* clinical isolates ($P < 0.001$)

vacA subtype	No. (%) of isolates	
	cagA$^+$	*cagA* negative
s1a	131 (99)	4 (21)
s2	1 (1)	15 (79)
Total	132 (100)	19 (100)

gene was detected in 132 (87%) of them. Concerning *vacA* subtypes, the s1a/m1 allelic combination was the most prevalent (65%), whereas combinations of s1a/m2 and s2/m2 were found in 24 and 11% of the cases, respectively.

The presence of the *cagA* gene correlated with that of *vacA* signal sequence type s1a, whereas type s2 was predominantly found in *cagA*-negative strains ($P < 0.001$) (Table 1). The relationships between *cagA* status, *vacA* subtypes, and disease in patients are shown in Table 2. In CG and PUD patients, all *cagA*-negative isolates were associated with the s2/m2 genotype. In contrast, for the PPU group, four *cagA*-negative isolates exhibited the *vacA* genotype s1a and conversely, one *cagA*-positive isolate exhibited the *vacA* genotype s2. However, statistically the PPU group results do not differ from those of the CG and PUD groups ($P > 0.05$), with the results of all three demonstrating the same correlation between *cagA* positivity and *vacA* s1a type.

This paper provides a comparison of the distributions of *H. pylori cagA* status and *vacA* genotypes in selected Estonian patients with confirmed diagnoses of CG, PUD, and PPU. Different consensus criteria for the role of *H. pylori* have been set for populations with high and low prevalences of *H. pylori* infections (11). We attempted to determine whether the same set of criteria was valid for a population with a high prevalence of *H. pylori*. In Estonia, according to serological studies, *H. pylori* prevalences are 87% in adults and 56% in children (28, 30).

In a study by Vorobjova et al. (29), high prevalences of the CagA protein in sample populations of Estonian adults (63%) and children (46%) have been demonstrated. In the present study, we found that the prevalence of the *cagA* gene among the large sample of patients with various clinical diagnoses was very high (87%). This corresponds to the findings of a previous study, which described a high (82%) prevalence of the *cagA* gene in cases of clinically complicated PUD (20). In addition, the present study hints that the *cagA* gene of *H. pylori* and its defined protein are important predictors of several different gastric diseases, even in populations with a high prevalence of infection.

In countries with a low prevalence of *cagA* positivity (1, 2, 18), a significant correlation between *vacA* subtypes and *cagA* status has been demonstrated. In our study of Estonian patients, a significant association between *vacA* subtypes and *cagA* status was also found. In particular, 87% of the *cagA*-positive strains had the *vacA* s1a/m1/m2 subtype and only 1 strain out of the 131 *cagA*-positive strains carried the *vacA* s2/m2 subtype.

According to a large number of studies (1, 2, 4, 7, 24), the *vacA* s1a/m1 subtype is considered more cytotoxic than the s1a/m2 and s2/m2 *vacA* genotypes and is therefore related to the development of PUD. The s2 strains are rarely associated with PUD and are more common in cases of CG or nonulcer dyspepsia (5, 7, 18, 22, 25). In our study, there was no difference in colonization by *H. pylori* cytotoxic strains between patients with peptic ulcer and those with the more severe PPU. Furthermore, few *H. pylori* strains were of the s2 subtype (6% in PUD patients and 10% in PPU patients). It could be speculated that, in these patients, the patchy distribution of gastric mucosa obscures the s1a strain, whereas the s2 strains are cocolonizers of the mucosa. Such colonization of the gastric mucosa with several different *H. pylori* strains has been described previously in numerous reports (8, 10, 17, 23). The *cagA*-positive strains from the CG patient samples exclusively carried the s1a subtype of the *vacA* gene. Within this group, however, the proportion of *cagA*-negative and *vacA* s2 alleles was the highest (20%) of all the disease groups. Therefore, the unusual result may depend on the severity of gastritis in these patients.

One important finding regarding the PPU group is that a small number of patients (8%) were apparently colonized by a *cagA*-negative strain that simultaneously expressed the *vacA* s1a subtype. Consequently, the possibility cannot be excluded that additional virulence markers of *H. pylori* contribute to the development of PPU in some patients with PUD, indicating a role for microbial as well as host genetic diversity.

The present study has also confirmed the findings of a previous investigation on geographic differences in the distribution of *H. pylori* strains (20). In the extended sample of 151 patients examined in the present investigation, no s1b subtype was detected. Our findings are similar to those for a population in Asia (*Helicobacter pylori* Study Group, Abstr. 9th Int. Workshop Gastroduodenal Pathol. Helicobacter, GUT **39**(Suppl. 2):A73–A74, 1996) and one in Poland, for which Gościniak et al. (9) found only 1 of 72 strains with the s1b type. Findings

TABLE 2. Relationship between *H. pylori cagA* status, *vacA* subtypes, and disease of patient group ($P > 0.05$)

vacA subtype	No. (%) of isolates in patients with:					
	CG ($n = 35$)		PUD ($n = 66$)		PPU ($n = 50$)	
	cagA$^+$ ($n = 29$)	*cagA* negative ($n = 6$)	*cagA*$^+$ ($n = 62$)	*cagA* negative ($n = 4$)	*cagA*$^+$ ($n = 41$)	*cagA* negative ($n = 9$)
s1a/m1	23 (79)	0	43 (69)	0	29 (71)	3 (33)
s1a/m2	6 (21)	0	19 (31)	0	11 (27)	1 (11)
s2/m2	0	6 (100)	0	4 (100)	1 (2)	5 (56)
Total	29 (100)	6 (100)	62 (100)	4 (100)	41 (100)	9 (100)

very different from ours have been established in several studies conducted in The Netherlands, Portugal, Brazil, Mexico, and the United States (1, 5, 17, 24). Our findings relate to the widely disparate evolutions of circulating *H. pylori* strains in a particular geographic area and the subsequent necessity for area-specific diagnostics for *H. pylori* virulence markers.

In conclusion, we found a high prevalence of the *cagA* gene of *H. pylori* within the population of strains studied. However, no clear differences existed in the distributions of *cagA* and *vacA* genotypes in Estonia between patients with severe PPU, uncomplicated PUD, or CG.

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REFERENCES

1. **Atherton, J. C., P. Cao, R. M. Peek, M. K. R. Tummuru, M. J. Blaser, and T. L. Cover.** 1995. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. J. Biol. Chem. **270:**17771–17777.
2. **Atherton, J. C., R. M. Peek, K. T. Tham, T. L. Cover, and M. J. Blaser.** 1997. Clinical and pathological importance of heterogeneity in *vacA*, the vacuolating cytotoxin gene of *Helicobacter pylori*. Gastroenterology **112:**92–99.
3. **Cover, T. L., and M. J. Blaser.** 1995. *Helicobacter pylori*: a bacterial cause of gastritis, peptic ulcer disease, and gastric cancer. ASM News **61:**21–26.
4. **Cover, T. L., M. K. R. Tummuru, P. Cao, S. A. Thompson, and M. J. Blaser.** 1994. Divergence of genetic sequences for the vacuolating cytotoxin among *Helicobacter pylori* strains. J. Biol. Chem. **269:**10566–10573.
5. **De Gusmão, V. R., E. N. Mendes, D. M. De Magalhaës Queiroz, G. A. Rocha, A. M. C. Rocha, A. A. R. Ashour, and A. S. T. Carvalho.** 2000. *vacA* genotypes in *Helicobacter pylori* strains isolated from children with and without duodenal ulcer in Brazil. J. Clin. Microbiol. **38:**2853–2857.
6. **The EUROGAST Study Group.** 1993. Epidemiology of, and risk factors for, *Helicobacter pylori* infection among 3,154 asymptomatic subjects in 17 populations. Gut **34:**1672–1676.
7. **Evans, D. G., D. M. M. Queiroz, E. N. Mendes, and D. J. Evans, Jr.** 1998. *Helicobacter pylori cagA* status and s and m alleles of *vacA* in isolates from individuals with a variety of *H. pylori*-associated gastric diseases. J. Clin. Microbiol. **36:**3435–3437.
8. **Go, M. F., V. Kapur, D. Y. Graham, and J. M. Musser.** 1996. Population genetic analysis of *Helicobacter pylori* by multilocus enzyme electrophoresis: extensive allelic diversity and recombinational population structure. J. Bacteriol. **178:**3934–3938.
9. **Gościniak, G., A. Gaszewska-Mastalarz, A. Przondo-Mordarska, J. Zakrzewska-Czerwińska, B. Iwańczak, and E. Poniewierka.** 1999. Diversity of *Helicobacter pylori vacA* gene and cytotoxin production. Clin. Microbiol. Infect. **5:**662–667.
10. **Jorgensen, M., G. Daskalopoulos, V. Warburton, H. M. Mitchell, and S. L. Hazell.** 1996. Multiple strain colonization and metronidazole resistance in *Helicobacter pylori*-infected patients: identification from sequential and multiple biopsy specimens. J. Infect. Dis. **174:**631–635.
11. **Lee, A.** 1999. *Helicobacter pylori*: opportunistic member of the normal microflora or agent of communicable disease?, p. 128–163. *In* G. W. Tannock (ed.), Medical importance of the normal microflora. Kluwer Academic Publishers, London, England.
12. **Maaroos, H.-I.** 1989. *Campylobacter pylori* in chronic gastritis and peptic ulcer. Acta Comment. Univ. Tartuensis **854:**59–68.
13. **Maaroos, H.-I.** 1995. *Helicobacter pylori* infection in the Estonian population: is it a health problem? Ann. Med. **27:**613–616.
14. **Maaroos, H.-I., T. Vorobjova, P. Sipponen, R. Tammur, R. Uibo, T. Wadström, R. Keevallik, and K. Villako.** 1999. An 18-year follow-up study of chronic gastritis and *Helicobacter pylori*: association of CagA positivity with development of atrophy and activity of gastritis. Scand. J. Gastroenterol. **34:**864–869.
15. **Marshall, B. J.** 1986. *Campylobacter pyloridis* and gastritis. J. Infect. Dis. **153:**650–658.
16. **Misiewicz, J. J., G. N. J. Tytgat, C. S. Goodwin, et al.** 1990. The Sydney system: a new classification of gastritis, p. 1–10. *In* World Congress on Gastroenterology Working Party Reports. Blackwell Science Publishing, Sydney, Australia.
17. **Morales-Espinosa, R., G. Castillo-Rojas, G. Gonzalez-Valencia, S. Ponce de León, A. Cravioto, J. C. Atherton, and Y. López-Vidal.** 1999. Colonization of Mexican patients by multiple *Helicobacter pylori* strains with different *vacA* and *cagA* genotypes. J. Clin. Microbiol. **37:**3001–3004.
18. **Rudi, J., A. Rudy, M. Maiwald, D. Kuck, A. Sieg, and W. Stremmel.** 1999. Direct determination of *Helicobacter pylori vacA* genotypes and *cagA* gene in gastric biopsies and relationship to gastrointestinal diseases. Am. J. Gastroenterol. **94:**1525–1531.
19. **Sillakivi, T., A. Tein, and A. Peetsalu.** 1999. Changing incidence and surgical management of perforated peptic ulcer (PPU) in Tartu county, Estonia, 1984–1997. Ann. Chir. Gynaecol. **88:**168.
20. **Sillakivi, T., H. Aro, M. Ustav, M. Peetsalu, A. Peetsalu, and M. Mikelsaar.** 2001. Diversity of *Helicobacter pylori* genotypes among Estonian and Russian patients with perforated peptic ulcer, living in Southern Estonia. FEMS Microbiol. Lett. **195:**29–33.
21. **Sipponen, P.** 1997. *Helicobacter pylori* gastritis—epidemiology. J. Gastroenterol. **32:**273–277.
22. **Strobel, S., S. Bereswill, P. Balig, P. Allgaier, H.-G. Sonntag, and M. Kist.** 1998. Identification and analysis of a new *vacA* genotype variant of *Helicobacter pylori* in different patient groups in Germany. J. Clin. Microbiol. **36:**1285–1289.
23. **Taylor, N. S., J. G. Fox, N. S. Akopyants, D. E. Berg, N. Thompson, B. Shames, L. Yan, E. Fontham, F. Janney, F. M. Hunter, and P. Correa.** 1995. Long-term colonization with single and multiple strains of *Helicobacter pylori* assessed by DNA fingerprinting. J. Clin. Microbiol. **33:**918–923.
24. **van Doorn, L. J., C. Figueiredo, R. Rossau, G. Jannes, M. van Asbroeck, J. C. Sousa, F. Carneiro, and W. G. V. Quint.** 1998. Typing of *Helicobacter pylori vacA* gene and detection of *cagA* gene by PCR and reverse hybridization. J. Clin. Microbiol. **36:**1271–1276.
25. **van Doorn, L. J., C. Figueiredo, R. Sanna, A. Plaisier, P. Schneebergen, W. A. de Boer, and W. G. V. Quint.** 1998b. Clinical relevance of the *cagA*, *vacA*, and *iceA* status of *Helicobacter pylori*. Gastroenterology **115:**58–66.
26. **van Doorn, L.-J., C. Figueiredo, R. Sanna, M. J. Blaser, and W. G. V. Quint.** 1999. Distinct variants of *Helicobacter pylori cagA* are associated with *vacA* subtypes. J. Clin. Microbiol. **37:**2306–2311.
27. **van Doorn, L.-J., Y. Henskens, N. Nouhan, A. Verschuuren, R. Vreede, P. Herbink, G. Ponjee, K. van Krimpen, R. Blankenburg, J. Scherpenisse, and W. Quint.** 2000. The efficacy of laboratory diagnosis of *Helicobacter pylori* infections in gastric biopsy specimens is related to bacterial density and *vacA*, *cagA*, and *iceA* genotypes. J. Clin. Microbiol. **38:**13–17.
28. **Vorobjova, T., H. Grünberg, M. Oona, H.-I. Maaroos, I. Nilsson, T. Wadström, A. Covacci, and R. Uibo.** 2000. Seropositivity to *Helicobacter pylori* and CagA protein in schoolchildren of different ages living in urban and rural areas in southern Estonia. Eur. J. Gastroenterol. Hepatol. **12:**97–101.
29. **Vorobjova, T., I. Nilsson, K. Kull, H.-I. Maaroos, A. Covacci, T. Wadström, and R. Uibo.** 1998. CagA protein seropositivity in a random sample of adult population and gastric cancer patients in Estonia. Eur. J. Gastroenterol. Hepatol. **10:**41–46.
30. **Vorobjova, T., K. Kisand, A. Haukanõmm, H.-I. Maaroos, T. Wadström, and R. Uibo.** 1994. The prevalence of *Helicobacter pylori* antibodies in a population from southern Estonia. Eur. J. Gastroenterol. Hepatol. **6:**529–533.

VII

Sillakivi T, Peetsalu A.
Perforeerunud peptilise haavandi riskifaktorid.
Eesti Arst 2000; 12: 718–722.

Eesti Arst 2000; 79(12): 718—722

Perforeerunud peptilise haavandi riskifaktorid

Toomas Sillakivi, Ants Peetsalu – Tartu Ülikooli kirurgiakliinik

perforeerunud peptiline haavand, *H. pylori*, riskifaktorid

Perforeerunud peptilise haavandi (PPH) epidemioloogia on viimastel aastakümnetel oluliselt muutunud. Arenenud riikides on PPH esinemissagedus vähenenud noortel patsientidel ja meestel ning suurenenud eakatel patsientidel ja naistel (1), seejuures on kasvanud PPH-ga patsientide keskmine vanus, naiste osakaal, mittesteroidsete põletikuvastaste ainete (MSPVA) tarvitajate ning kaasuvate haigustega patsientide arv (2, 3). PPH kirurgilises ravis on toimunud muutused mittedefinitiivsete operatsioonide kasuks (4). Sellisele ravitaktikamuutusele on kõige olulisemat mõju avaldanud postoperatiivne *Helicobacter pylori* eradikatsioonile suunatud ravi.

Tartu piirkonnas on aastatel 1991–97 (võrreldes perioodiga 1978–90) aset leidnud järsk PPH esinemissageduse kasv, sarnane tendents on olnud omane õigupoolest kogu Eestile. Samas ei ole oluliselt muutunud PPH-ga patsientide vanus ega sooline koosseis (5). PPH esinemissagedus aastatel 1991–97 (16–26 juhtu 100 000 inimese kohta aastas) ületab 2–10 korda viimaste aastakümnendite vastavaid näitajaid (2,3–10 juhtu 100 000 inimese kohta aastas) arenenud riikides (3, 6, 7).

Selle uuringu eesmärgiks oli välja selgitada PPH suure esinemissageduse võimalikke põhjuseid Eesti patsientidel. Püüdsime hinnata PPH riskifaktorite esinemist, tüsistusele eelnenud diagnostikat ning praegusaegsetele arusaamadele vastavat haavandiravi nendel patsientidel enne perforatsiooni teket.

Uurimismaterjal ja –meetodid

Prospektiivne uuring hõlmas kõiki ajavahemikul 1. jaanuarist 1997. a kuni 31. detsembrini 1999. a Tartu Maarjamõisa Haiglasse hospitaliseeritud 129 PPH-ga patsienti. Iga patsiendi kohta täideti pre- ja postoperatiivselt spetsiaalsed protokollid, mis hõlmasid ka põhjalikku elu- ja haiguse anamneesi, sealhulgas võimalikke PPH riskifaktoreid (stress, patsiendi sotsiaalne staatus, suitsetamine, mittesteroidsete põletikuvastaste ainete kasutamine ning eelneva haavandtõveravi kasutamine või mittekasutamine), millele on viidatud eelnevates uuringutes (1, 8, 9). Tüsistusele eelnenud perioodil patsiendile tehtud haavndtõveravi klassifitseeriti järgmiselt: ravi vajaduse korral (vaevuste tekkel ravimite tarvitamine mõne päeva jooksul), vahelduvravi (1–2 kuud kestev ravi haiguse ägenemise ajal) ning säilitusravi (ravimite igapäevane tarvitamine vähemalt viimase 3 kuu jooksul) (10). *H. pylori* identifitseerimiseks koguti patsientide juhusliku valiku alusel 2 antrumi ja 2 maokorpuse limaskesta proovitükki kas operatsiooni ajal biopsiatangidega läbi perforatsiooniava või postoperatiivselt panendoskoopial. *H. pylori* esinemist hinnati 96 juhul histoloogiliselt, kasutades Giemsa värvingut, ning 53 juhul täiendavalt antrumi limaskesta biopsiamaterjali uurides PCR (polümeraasahelreaktsioon) metoodika abil (11). Väljalõigatud haavandit või haavandi servast võetud proovitükki uuriti histoloogiliselt hematoksüliineosiinvärvingut kasutades 77 patsiendil.

Tulemused

Prospektiivse uuringu perioodil aastatel 1997–99 hospitaliseeriti Tartu Maarjamõisa haiglasse 129 PPH-ga patsienti keskmise vanusega 48,6 aastat (96 meest keskmise vanusega 42,8 a ja 33 naist keskmise vanusega 65,6 a). 111 juhul esines

duodenaalhaavandi ja 16 juhul maohaavandi perforatsioon. Arvestades ainult Tartu piirkonda sisse kirjutatud patsiente, olid PPH esinemissageduse näitajad aastatel 1997–99 vastavalt 26, 17 ja 22 juhtu 100 000 inimese kohta aastas.

Ainult 30%-l patsientidest oli haavandtõbi diagnoositud enne perforatsiooni teket (12% endoskoopiliselt, 4% röntgenoloogiliselt, 12% mõlema meetodi abil ning 2% varasemal perforatsiooni tõttu tehtud operatsioonil) (vt jn 1). Aeg haavandi diagnoosimisest kuni perforatsiooni tekkeni varieerus 2 kuust kuni 45 aastani (mediaan 8 aastat). Lisaks oli veel 53%-l patsientidest eelnevalt esinenud haavandtõvele iseloomulikke ülakõhukaebusi. Seejuures 78% kõigist patsientidest ja 44% nendest, kellel haavandtõbi oli eelnevalt diagnoositud, ei olnud viimase aasta jooksul haavandtõveravimeid tarvitanud (vt jn 2), ainult 1 patsient (3%) oli tarvitanud omeprasooli. Mitte ükski 129 patsiendist polnud saanud *H. pylori* eradikatsioonile suunatud ravi. 11% patsientidest tarvitas pidevalt MSPVA-d (vt jn 3). 77% patsientidest olid suitsetajad ning 63% tunnistas stressi esinemist (patsiendi enda subjektiivne hinnang) (vt jn 4). 24% patsientidest olid pensionärid, 41% töötasid ning 20% olid töötud või elatusid juhutöödest.

Histoloogiliselt (Giemsa värving) uuritud maolimaskestatükkides oli 97%-l juhtudest (93/96) sedastatav *H. pylori* esinemine, seda tõestasid ka PCR (polümeraasahelreaktsioon) metoodikal tehtud antrumi limaskestauuringud – *H. pylori* oli sedastatav 96%-l juhtudest (51/53). Kõigil 77 patsiendil, kellel uuriti haavandit histoloogiliselt, oli tegemist kroonilise haavandi perforatsiooniga.

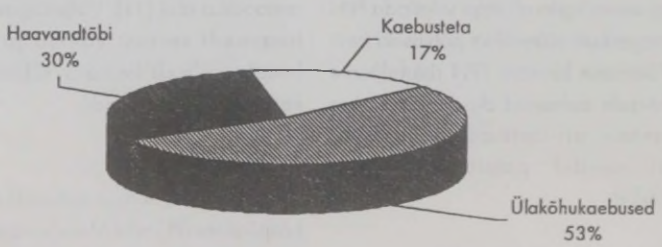
Arutelu

Sarnaselt meie retrospektiivse uuringuga (5) olid ka selle prospektiivse uuringu patsiendid oluliselt nooremad (keskmine vanus 48 aastat *vs* 53–63 a lääneriikides) ning naiste osakaal väiksem (26% *vs* 40–69% lääneriikides) kui enamikus arenenud riikides tehtud uuringutes (2, 3, 6, 12). Seevastu on patsientide jaotus meie uuringus ja 1997. a Ida-Euroopa riikides tehtud uuringus (Copernicuse programm – ägeda kõhuvalu uuring Ida-Euroopas) sarnased (13).

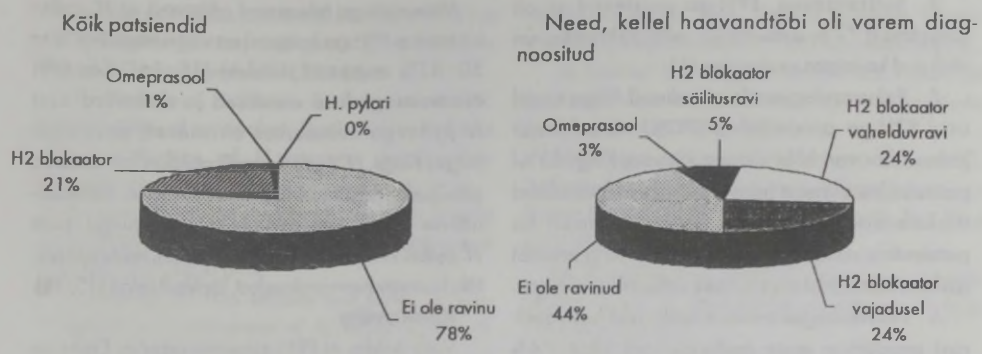
Selle uuringu tulemused viitavad mitmetele faktoritele, mille koosmõju on ilmselt viinud järsule PPH esinemissageduse kasvule, ning sellele, miks viimase aastakümnendi näitajad arenenud riikidega võrreldes on sedavõrd suured.

1. Ühiskondliku stressi osa. Sarnaselt PPH esinemissageduse kõvera järsu tõusuga aastast 1991 on käitunud ka suitsiidikõver Eestis (14). Analoogiline PPH esinemissageduse oluline kasv esines ka Hong-Kongis pärast iseseisvumist, mil tõsiste poliitilis-majanduslike muutuste foonil märgati ka stressi suurenemist ühiskonnas (8).

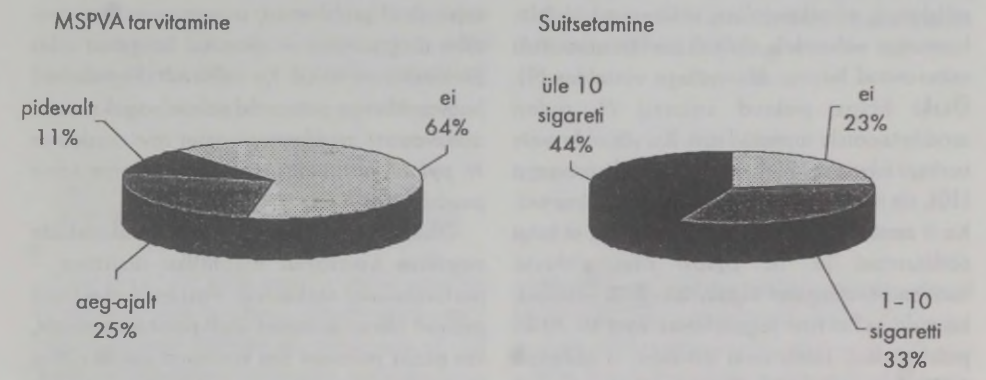
2. Sotsiaalsed faktorid. Uuritud PPH-ga patsientide seas oli rohkelt nn madalama sotsiaalse klassi esindajaid (ainult 41%-l oli stabiilne töökoht).



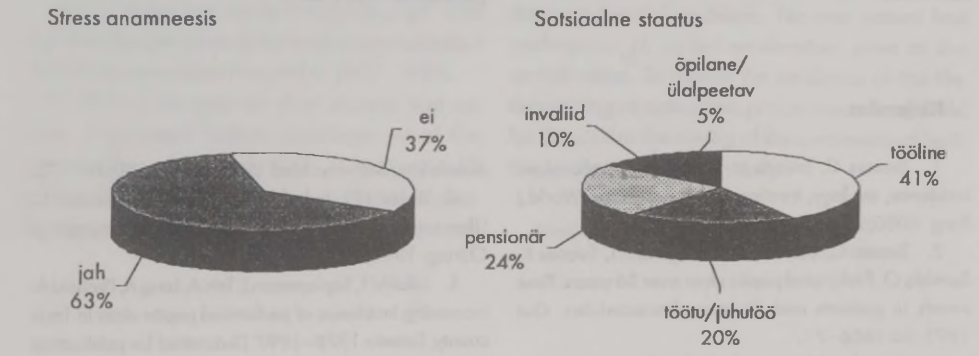
Joonis 1. PPH-ga patsientide osakaal, kellel on varem diagnoositud haavandtõbi või esinenud ülakõhukaebused



Joonis 2. Haavandtõve varasem ravi PPH-ga patsientidel



Joonis 3. Mittesteroidsete põletikuvastaste ainete (MSPVA) tarvitamine ja suitsetamine PPH-ga patsientide hulgas



Joonis 4. Stress ja sotsiaalne staatus PPH-ga patsientidel

3. Suitsetamine. PPH-ga patsientidest oli suitsetajaid 77%; suitsetamise rollile PPH tekkes on viidatud ka teistes uuringutes (1).

4. Eelneva diagnostika puudused. Väga suurel osal PPH-ga patsientidest (70%) oli eelnevalt jäänud haavandtõbi diagnoosimata, kuigi 53%-l patsientidest esinesid haavandtõvele iseloomulikud ülakõhukaebused. Põhjuseks on ilmselt ka patsientide vähene haigusteadlikkus ning arstiabi raskem kättesaadavus mõnes sotsiaalses rühmas.

5. Nüüdisaegse medikamentoosse haavandiravi puudumine enne perforatsiooni teket. 44% haavandidiagnoosiga patsientidest polnud perforatsioonile eelnenud aasta jooksul saanud haavandiravi. Varasemates uuringutes on näidatud, et pikaajaline säilitusravi H_2-blokaatoriga vähendab oluliselt perforatsiooniriski mitteravitud haavandihaigetega võrreldes (9). Ükski haige polnud saanud *H. pylori* eradikatsioonile suunatud ravi. Kui võrrelda meie uuringu tulemusi 1995. a Eestis tehtud uuringuga (10), siis näeme, et olulist muutust pole toimunud. Ka 5 aastat tagasi tehtud küsitlus näitas, et kuigi säilitusravi ja *H. pylori* ravi pidasid haavandtõvehaigetel õigeks 34–84% arstidest, kasutati sellist ravi tegelikkuses vaid 0–20%-l patsientidest. Tuleb siiski märkida, et erinevalt 1995. aastal arstide küsitluse teel saadud andmetest oleme meie oma uuringus hinnanud patsientidelt saadud andmeid reaalselt kasutatud ravi kohta.

Meie uuringu tulemused näitavad, et *H. pylori* esinemine PPH-ga haigetel on väga sage (97% *vs* 50–83% arenenud riikides) (15, 16). Kas PPH esinemissageduse muutused ja sedavõrd suur *H. pylori*'ga infitseeritus on seotud, pole siiski selge. Kuigi *H. pylori* eradikatsioon on saanud põhiliseks argumendiks haavandtõve mittedefinitiivse operatiivse ravitaktika pealetungil, pole *H. pylori* roll perforatsioonide puhul, erinevalt näiteks haavandiverejooksudest, lõplikult selge (15, 16).

Kokkuvõte

Võib öelda, et PPH esinemissagedus Eestis on viimasel kümnendil jätkuvalt väga suur. Meie patsientidel esinevate riskifaktorite seast võiks välja tuua *H. pylori* suure esinemissageduse, stressi, sotsiaalsed probleemid, suitsetamise. Haavandtõbe diagnoositakse enamikul haigetest alles perforatsiooni tekkel. Ka eelnevalt diagnoositud haavandtõvega patsiendid polnud sageli saanud adekvaatset medikamentoosset ravi, kusjuures *H. pylori* eradikatsiooni rakendamine ravis puudub üldse.

Olukorra parandamiseks tuleb vähendada peptilise haavandi eluohtliku tüsistuse – perforatsiooni tekkeohtu. Vastavad meetmed peavad olema suunatud ühelt poolt arstkonnale, kes peaks paremini ära kasutama haavandtõve tänapäeva ravivõimalusi, ja teiselt poolt tuleb tõhustada selgitustööd inimeste seas, et suurendada haigusteadlikkust ning julgustada pöörduma arsti poole.

Kirjandus

1. Svanes C. Trends in perforated peptic ulcer: incidence, etiology, treatment, and prognosis. World J Surg 2000;24:277–83.

2. Svanes C, Salvesen H, Stangeland L, Svanes K, Søreide O. Perforated peptic ulcer over 56 years. Time trends in patients and disease characteristics. Gut 1993;34:1666–71.

3. Aeberhard P, Lichtenhahn P, Villiger P. Heutiger Stand der Therapie des perforierten Gastroduodenalulkus. Schweiz Med Wschr 1990;120:467–75.

4. Roher HD, Imhof M, Goretzki PE, Ohmann C. Ulcer surgery '96 – choice of methods in an emergency. Chirurg 1996;67: 20–5.

5. Sillakivi T, Soplepmann J, Tein A, Lang A, Peetsalu A. Increasing incidence of perforated peptic ulcer in Tartu county, Estonia 1978–1997. (Submitted for publication in Hepatogastroenterol).

6. Hermansson M, Stael von Holstein C, Zilling T.

Peptic ulcer perforation before and after the introduction of H_2-receptor blockers and proton pump inhibitors. Scand J Gastroenterol 1997;32:523-9.

7. Makela J, Laitinen S, Kairaluoma MI. Complications of peptic ulcer disease before and after the introduction of H_2-receptor antagonists. Hepatogastroenterol 1992;39:144-8.

8. Lam SK, Hui WM, Shiu LP, Ng MM. Society stress and peptic ulcer perforation. J Gastroenterol Hepatol 1995;10:570-6.

9. Penston JG. The efficacy and safety of long-term maintenance treatment of duodenal ulcers with ranitidine. Scand J Gastroenterol 1990;25 (Suppl 177):42-51.

10. Labotkin K. Haavandtõve medikamentoosne ravi Eestis. Eesti Arst 1996;(5):387-92.

11. Sillakivi T, Aro H, Ustav M, Peetsalu M, Peetsalu A, Mikelsaar M. Diversity of *Helicobacter pylori* genotypes among Estonian and Russian patients with perforated peptic ulcer, living in Southern Estonia. (Accepted for publication in FEMS Microbiol Letters).

12. Hamby LS, Zweng TN, Strodel WE. Perforated gastric and duodenal ulcer: an analysis of prognostic factors. Am Surg 1993;59:319-24.

13. Sillakivi T, Yang Q, Peetsalu A, Ohmann C. Perforated peptic ulcer: Is there a difference between Eastern Europe and Germany? Langenbeck's Arch Surg 2000;385:344-9.

14. Wasserman D, Värnik A. Increase in suicide among men in the Baltic countries. Lancet 1994;343:1504-5.

15. Reinbach DH, Cruckshank G, McColl KEL. Acute perforated duodenal ulcer is not associated with *Helicobacter pylori* infection. Gut 1993;34:1334-7.

16. Sebastian M, Chandran VP, Elashaal YI, Sim AJ. *Helicobacter pylori* infection in perforated ulcer disease. Br J Surg 1995;82:360-2.

Summary

Risk factors of perforated peptic ulcer

The aim of the study was to clarify possible causes of the high incidence of perforated peptic ulcer in Estonian patients by assessment of risk factors, including pre-complication diagnostics and use of ulcer treatment, on the basis of contemporary knowledge, in these patients before development of perforation.

The prospective study comprised all 129 perforated peptic ulcer patients who were admitted to Tartu Maarjamõisa Hospital in 1997-1999.

In 70% of our patients ulcer disease had not been diagnosed before development of the complication, and even 44% of patients with previously proved ulcer diagnosis had not taken drugs against ulcer disease (H_2- blockers, proton pump inhibitors). No patient had passed treatment for *Helicobacter pylori*. Of the patients 77% were smokers, 63% mentioned stress in everyday life and in 97% *H. pylori* infection was detected in the gastric mucosa. The high incidence of perforated peptic ulcer can be related to high *H. pylori* infection among our patients as well as to frequent smoking, stress and social problems. No one patient had undergone *H. pylori* eradication prior to the complication. To reduce the incidence of this life-threatening complication, proper measures should be directed to the raising of the awareness of both the medical staff and patients.

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VIII

Sillakivi T, Peetsalu M, Mikelsaar M, Peetsalu A.
Helicobacter pylori eradikatsioon perforeerunud peptilise haavandiga haigetel. Eesti
Arst 2001; 1: 8–11.

Eesti Arst 2001; 80(1): 8—11

Helicobacter pylori eradikatsioon perforeerunud peptilise haavandiga haigetel

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Tartu piirkonnas (aga ka kogu Eestis) on alates 1991. a järsult suurenenud perforeerunud peptilise haavandi (PPH) esinemissagedus, kusjuures *H. pylori* esinemine meie PPHga patsientide maolimaskestas ulatub 96–97%-ni (1).

PPH kirurgilise ravi taktika on kogu maailmas muutunud mittedefinitiivsete operatsioonide (s.o perforeerunud haavandi üleõmblus ja ekstsisioon) kasuks, mida on suures osas põhjendatud just *H. pylori* postoperatiivse eradikatsioonivõimaluse lisandumisega (2). Haavandiverejooksude korral on *H. pylori* eradikatsioon andnud positiivseid tulemusi: on vähenenud verejooksu ja haavandi retsidiivi võimalus (3, 4). Perforatsioonide puhul pole aga *H. pylori* eradikatsiooni efektiivsus senini üheselt selge.

Pikaajalised uuringud (patsiente on jälgitud kuni 14 a pärast operatsiooni) on näidanud, et peaaegu kõigil duodenaalhaavandiga haigetel on mao limaskest *H. pylori* infektsioonist haaratud ning vagotoomia kui efektiivne ravimeetod, mis vähendab küll happe-peptilist faktorit, ei elimineeri *H. pylori* kolonisatsiooni maost ning retsidiivhaavandite arv kasvab aja jooksul pidevalt pärast operatiivset ravi (5). Seepärast oleks otstarbekas operatiivse ravi ajal või selle järel PPHga haigetel teha ka *H. pylori*'t elimineeriv ravikuur, et vähendada retsidiivhaavandite teket. Kirjanduse andmetel on sellist taktikat proovitud ainult üksikutel juhtudel, kuigi *H. pylori* eradikatsiooni tulemused on olnud paljulubavad. Nii jälgis Friess kaasautoritega (6) tüsistumata duodenaalhaavandiga haigetel *H. pylori* eradikatsiooni efektiivsust ühekordse operatsiooniaegse antibiootikumprofülaktika järel (meslotsilliin ja metronidasool) ning saavutas pärast 3-kuulist jälgimisaega *H. pylori* eradikatsiooni 85%-l haigetest. Ng kaasautoritega (7) kasutas PPHga haigetel vahetus postoperatiivses perioodis peroraalset nelikravi (vismut subtsitraat, tetratsükliin, metronidasool ning omeprasool) ning sai hea eradikatsioonitulemuse (83%).

Käesoleva uuringu eesmärgiks oli leida PPHga patsientidele Eestis *H. pylori* eradikatsiooniks efektiivne postoperatiivne raviskeem.

Uurimismaterjal ja –meetodid

Tartu Maarjamõisa Haiglas korraldati aastatel 1997–1999 prospektiivne uuring, kus PPHga patsientidelt koguti operatsiooni ajal biopsiatangidega läbi perforatsiooniava 2 antrumi ja 2 maokorpuse limaskestabioptaati *Helicobacter pylori* identifitseerimiseks. *H. pylori* eradikatsiooniks kasutati 6 erinevat raviskeemi 49 mittejärjestikusel patsiendil kas vahetult postoperatiivselt haiglas (intravenoosne 5-päevane ravi) või ambulatoorselt 7–10 päeva möödumisel operatsioonist (peroraalne 7-päevane ravi) (vt tabel 1). Kaks peroraalset kolmikraviskeemi (I ja II), mida me kasutasime mittevagotomeeritud patsientidel, on maailmas laialt levinud, need on nn klassikalised kombinatsioonid: amoksitsilliini 1000 mg *b.i.d.* + metronidasooli 500 mg *q.i.d.* + omeprasooli 20 mg *b.i.d.* (8 patsienti) ning amoksitsilliini 1000 mg *b.i.d.* + klaritromütsiini 500 mg *b.i.d.* + omeprasooli 20 mg *b.i.d.* (11 patsienti). Viimati nimetatud raviskeemi on soovitanud ka Eesti Gastroenteroloogide Selts (8). Ülejäänud meie kasutatud raviskeemid (III–VI) olid modifitseeritud: neist amoksitsilliini 1000 mg *b.i.d.*

Tabel 1. *H. pylori* eradikatsiooni tulemused 2 kuu möödudes perforeerunud peptilise haavandiga patsientidel

	Ravi skeem	Haigete arv	Keskmine vanus (a)	Mehi / naisi	Duodenaal- / maohaavand	Eradikatsioon patsientide arv (%)
I	AKO* p.o. 7 päeva	11	40,0	11/0	9/2	2 (18,2)
II	AMO* p.o. 7 päeva	8	31,9	8/0	8/0	3 (37,5)
III	AM** p.o. 7 päeva	16	46,2	15/1	16/0	4 (25,0)
IV	AK** p.o. 7 päeva	6	37,7	5/1	6/0	1(16,7)
V	KLACID*** i.v. 5 päeva	6	54,0	5/1	5/1	0 (0)
VI	KLACID*** p.o. 7 päeva	2	54,0	1/1	1/1	0 (0)
VII	KONTROLLGRUPP***	10	46,1	8/2	6/4	0 (0)

A - amoksitsilliin (Amoxicillin-ratiopharm® 1 g b.i.d.

K - klaritromütsiin (Klacid®, Abbott) 0,5 g b.i.d.

M - metronidasool (Metronidazole BP®, Pharmachem) 0,5 g q.i.d.

O - omeprasool (Losec®, Astra) 20 mg b.i.d.

KONTROLLGRUPP - patsiendid, kellel *H. pylori* eradikatsiooni ei tehtud

* - mittevagotomeeritud patsiendid

** - ilma prootonpumba inhibiitorita ravitud patsiendid, kellel operatsioonil tehti ka trunkaalne vagotoomia

*** - nii vagotomeeritud kui ka vagotomeerimata patsiendid

+ metronidasooli 500 mg *q.i.d.* (16 patsienti) ning amoksitsilliini 1000 mg *b.i.d.* + klaritromütsiini 500 mg *b.i.d.* (6 patsienti) olid modifikatsioonid kahest eelmisest, mida kasutasime vagotomeeritud patsientidel, et kontrollida, kas vagotoomia võiks sellisel puhul asendada omeprasooli. Klaritromütsiini monoteraapiat (500 mg *b.i.d.*) rakendati perioodil, mil see ravim oli Eestis kasutusel alles esimesi nädalaid, eeldades, et *H. pylori* ei ole selle preparaadi suhtes resistentne. V grupis (6 patsienti) kestis intravenoosne ravi 5 päeva ja VI grupis peroraalne monoteraapia 7 päeva (2 patsienti) (vt tabel 1). Järelkontrollil 2–3 kuu möödumisel tehti panendoskoopia ning võeti limaskesta proovitükid (2 antrumist ja 2 korpusest). *H. pylori* hindamiseks kasutati Giemsa värvingut ning tulemust peeti positiivseks, kui *H. pylori* oli sedastatav vähemalt ühes preparaadis.

Tulemused

H. pylori eradikatsiooniks mõeldud ravi tulemused 49 PPHga patsiendil on esitatud tabelis 1. Histoloogilise hinnangu alusel saavutati *H. pylori* eradikatsioon erinevate raviskeemide kasutamisel 0 kuni 37,5% juhtudest, kusjuures kontrollrühmas (patsiendid, kes polnud saanud *H. pylori* vastast ravi) ei toimunud ühelgi patsiendil eradikatsiooni. 2 patsienti (ravi amoksitsilliini, metronidasooli ja omeprasooliga) katkestasid ravi kõrvaltoimete (diarröa) tõttu ning need patsiendid on tabelist ja analüüsist välja jäetud.

Arutelu

Vastavalt Maastrichti konsensusele 1996. a septembrist (9) on tänapäeval *H. pylori* eradikatsiooni soovitatud kõigile peptilise haavandiga patsientidele, kellel on tõestatud *H. pylori* olemasolu maolimaskestas. Eradikatsiooni tulemused peroraalsete kolmik- või nelikraviskeemide puhul ületavad 80–90% enamikus publitseeritud töödes (10, 11). Samas on väga vähe informatsiooni sellise taktika edukuse kohta PPHga patsientide puhul, sest enamasti on need patsiendid uuringutest välja jäetud (12).

Meie uuringus toimus *H. pylori* eradikatsioon oodatust oluliselt väiksemal osal PPHga patsientidest ja seda ka kahe maailmas laialt levinud klassikalise kolmikraviskeemiga: I raviskeem (amoksitsilliin, klaritromütsiin ning omeprasool), mida on soovitanud Eesti Gastroenteroloogide Selts (8), andis positiivse eradikatsioonitulemuse ainult 18,2%-l juhtudest; II raviskeem (metronidasool, amoksitsilliin ning omeprasool) andis positiivse eradikatsioonitulemuse 37,5%-l juhtudest (vt tabel 1).

Ka modifitseeritud raviskeemid ei andnud rahuldavat tulemust: eradikatsioon 0–25%-l juhtudest. Miks toimus *H. pylori* eradikatsioon meie uuringus niivõrd väikesel osal patsientidest, jääb ebaselgeks. Võimalike põhjustena tulevad arvesse järgmised asjaolud.

1. Meie PPHga patsientidel postoperatiivses perioodis tehtud ühenädalased peroraalsed ravikuurid, mis on Eestis andnud positiivseid tulemusi mittetüsistunud haavandihaigetel, ei sobi ilmselt PPH puhul või vajavad need haiged pikemat ravikuuri. Ometi on ravi amoksitsilliini, klaritromütsiini ja omeprasooliga soovitanud Eesti Gastroenteroloogide Selts ning see on andnud 91%-lise eradikatsioonitulemuse (13).

2. Liialt varane (7.–10. postoperatiivsel päeval) ravi alustamine meie PPHga patsientidel. Selle põhjuse vastu räägivad teiste uuringute tulemused, milles PPHga haigetel juba alates 3. postoperatiivsest päevast alustatud *H. pylori* ravi oli 83%-l juhtudest edukas (7).

3. Probleemid ravirežiimist kinnipidamisega. Seda on peetud üheks olulisemaks *H. pylori* eradikatsiooni ebaõnnestumise põhjuseks (10). Ambulatoorselt puudub vahetu kontrollimise võimalus ravimite tegeliku kasutamise üle. Kuna aga patsiendid ilmusid 2–3 kuu möödumisel ravikuurist korrektselt ambulatoorsele järelkontrollile, kus tehti ka patsiendi jaoks ebameeldiv protseduur – endoskoopia, siis viitab see haigepoolsele koostöövalmidusele ning *H. pylori* eradikatsioonist huvitatusele.

Meie uuringu tulemused näitavad, et PPHga haigetele sobiva efektiivsema raviskeemi leidmine vajab veel edaspidiseid uuringuid. *H. pylori* mikroobitüvede eripära selgitamine PPH puhul võiks pakkuda uusi lahendusi ka raviprobleemidele.

Kokkuvõtteks võib öelda, et kõik meie töös *H. pylori* eradikatsiooniks kasutatud raviskeemid, sealhulgas ka Eesti gastroenteroloogide soovitatud ravikombinatsioon, ei ole PPHga patsientidel eeldatud efekti andnud. PPH suure esinemissageduse ja nende patsientide 96–97%-lise *H. pylori*'ga infitseerituse taustal nõuab meie oludele sobiva ravitaktika väljatöötamine jätkuvaid uuringuid, et vältida postoperatiivselt retsidiivhaavandite ning nendest tingitud eluohtlike tüsistuste tekkimist.

Kirjandus

1. Sillakivi T, Peetsalu A. Perforeerunud peptilise haavandi riskifaktorid. Eesti Arst 2000;79(12):718–23.
2. Roher HD, Imhof M, Goretzki PE, Ohmann C. Ulcer surgery '96 – choice of methods in an emergency. Chirurg 1996;67:20–5.
3. Sonnenberg A, Olson CA, Zhang J. The effect of antibiotic therapy on bleeding from duodenal ulcer. Am J Gastroenterol 1999;94:950–4.
4. Laine L. The long-term management of patients with bleeding ulcers: *Helicobacter pylori* eradication instead of maintenance antisecretory therapy. Gastrointest Endosc 1995;41:77–9 (editorial).
5. Peetsalu M, Maaroos H-I, Peetsalu A. Completeness of vagotomy, *Helicbacter pylori* colonization and recurrent ulcer 9 and 14 years after operation in duodenal ulcer patients. Eur J Gastroenterol Hepatol 1998;10:305–11.
6. Friess H, Malfertheiner P, Flock F, Baczako K, Stanescu A, Büchler M. Elimination of *H. pylori* by single shot antibiotic treatment in patients undergoing proximal gastric vagotomy. Eur J Gastroenterol Hepatol 1992;4:219–25.
7. Ng EKW, Lam YH, Sung JJY, Yung MY, To KF, Chan ACW, et al. Eradication of *Helicobacter pylori* prevents recurrence of ulcer after simple closure of duodenal ulcer perforation. Ann Surg 2000;231:153–8.
8. Labotkin K, Maaroos H-I, Salupere R. *Helicobacter pylori* infektsiooni ravi juhend. Eesti Arst 1999;(3):280–2.
9. European Helicobacter Pylori Study Group: Current European Concepts on the Management of *Helicobacter Pylori* Infection. The Maastricht Consensus Report. 1996.
10. Freston JW. Management of peptic ulcers: Emerging issues. World J Surg 2000; 24:250–5.
11. Wermeille J, Zelger G, Cunningham M. The eradication regimens of *Helicobacter Pylori*. Pharmacy World Science 1998;20(1):1–17.
12. Sonnenberg A, Schwartz J, Sanford J, Cutler AF, Vakil N, Bloom B. Cost savings in duodenal theraphy through Helicobacter pylori eradication compared with conventional therapies: result of a randomized, double-blind, multicenter trial. Arch Int Med 1998;158:852–60.
13. Kolk H, Maaroos H-I, Labotkin K, Kull I, Lõivukene K, Mikelsaar M jt. *Helicobacter pylori* reinfektsioon. Arstiteaduskonna aastakonverentsi teesid; Tartu, 1998;29.

Summary

Eradication of *H. pylori* in perforated peptic ulcer patients

The aim of the study was to develop an effective postoperative regimen for *H. pylori* eradication in perforated peptic ulcer patients in Estonia.The treatment aimed at *H. pylori* eradication was applied in 49 perforated peptic ulcer patients in the postoperative period using 6 different treatment regimens, including 2 widely recognized triple regimens. The results of *H. pylori* eradication were assessed histologically. Gastric mucosal specimens were collected at the operation and after 2-3 months of treatment on panendoscopy.

The results of *H. pylori* eradication in our different treatment regimens varied from 0 to 37,5%. Considering the extremely high incidence of *H. pylori* infection and the hitherto unsatisfactory results of eradication, it is necessary to continue investigations aimed at working out an appropriate treatment regimen for perforated peptic ulcer patients in our conditions.

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Education

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1994–1996	Internship at Maarjamõisa Hospital, University of Tartu
1996– 2000	Doctoral student at the Department of Surgery, University of Tartu
2000–	Resident at the Department of Surgery, University of Tartu

Special courses

1998	Düsseldorf,H-Heine University, Clinic of Surgery, Germany, Copernicus Project Week,
1999	2rd Baltic Endoscopy Workshop Jurmala, Latvia,
2001	3rd Baltic Endoscopy Workshop, Vilnius, Lithuania

Professional employment

2000–	Senior laboratory assistant, Clinic of Surgery, University of Tartu

Scientific work

Research field:

1. Perforated peptic ulcer- epidemiology, risk factors and relations with *H.pylori*.
2. Acute abdominal pain (participation in international programme of acute abdominal pain EAS AAP)

15 scientific publications, 10 presentations at the international congresses (including 4 world or european surgical congresses).

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Erialane enesetäiendus

1998	Düsseldorfi H-Heine Ülikooli kirurgiakliinik, Saksamaa, Copernicus kirurgianädal
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2001	Kolmas Balti Endoskoopia Workshop, mai, 2001, Vilnius, Leedu

Erialane teenistuskäik

2001–	TÜ kirurgiakliinik, vanemlaborant

Teadustöö

Peamised uuringuteemad:

1. Perforeerunud peptiline haavand, epidemioloogia, ravi, seosed *H.pylori*ga
2. Äge kõhuvalu (osalemine rahvusvahelises kõhuvalu uurimise programmis EASAAP)

15 teaduspublikatsiooni, 10 ettekannet rahvusvahelistel kongressidel (sealhulgas 4 maailma või euroopa kirurgiakongressidel)

Eesti Kirurgide Assotsiatsiooni liige aastast 1999.

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