

Influence of age and *Helicobacter pylori* infection on serum pepsinogens in healthy blood transfusion donors

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Abstract

In a healthy population pepsinogen A and pepsinogen C increase with advancing age. As pepsinogen A and C are raised in chronic superficial gastritis which is caused by *H pylori* infection, we investigated whether *H pylori* is responsible for the age related increase of pepsinogen A and C. In *H pylori* positive blood transfusion donors serum pepsinogen A (mean (SD) 73 (35) $\mu\text{g/ml}$ v 52 (19) $\mu\text{g/ml}$, $p < 0.01$) and C (mean (SD) 24 (13) $\mu\text{g/ml}$ v 10 (7) $\mu\text{g/ml}$, $p < 0.01$) concentrations were significantly higher than in *H pylori* negative blood transfusion donors, while the serum pepsinogen A:C ratio (mean (SD) 3.5 (1.4) v 6.2 (3.4), $p < 0.01$) was significantly decreased because of a relative greater increase in serum pepsinogen C in *H pylori* positive blood transfusion donors. Analysis of variance showed that pepsinogen A and C concentrations differed significantly in the different age groups ($p < 0.01$) when we considered all blood transfusion donors and *H pylori* positive blood transfusion donors, the mean pepsinogen levels being highest in the older age categories. In *H pylori* negative blood transfusion donors no such age related difference in pepsinogen A and C could be shown. In *H pylori* positive blood transfusion donors a weak positive but significant correlation between pepsinogen A and C and age could be shown ($r = 0.30$; $p = 0.01$ and $r = 0.31$; $p = 0.01$ respectively). In *H pylori* negative blood transfusion donors no correlation between serum pepsinogens and age was found. We conclude that the age related increase in serum pepsinogen A and C described in healthy control populations is caused by an increasing prevalence of *H pylori* infection. Serum pepsinogen A and C concentrations in patients should therefore be related to the presence or absence of *H pylori* infection.

Pepsinogen A and C are the proenzymes of pepsin A and pepsin C, respectively, which belong to the group of gastric acid aspartic endoproteases. Pepsinogen A is secreted by the fundic mucosa¹ while pepsinogen C is secreted by fundic glands, pyloric glands, and proximal duodenal mucosa.²

Serum pepsinogen A and pepsinogen C concentrations can predict the histological status of the gastric mucosa and show also non-parallel changes in serum concentrations with increasing severity of gastritis^{3,4} showing a relatively greater

rise of pepsinogen C in patients with more severe gastritis. In healthy controls an increase in pepsinogen A and pepsinogen C serum concentrations with age has been described by several authors.⁵⁻⁷ The incidence of *H pylori* infection increases with age^{8,9} and *H pylori* is generally accepted as the cause of chronic superficial gastritis^{10,11} which can be a cause of increased serum pepsinogen A and C levels.^{3,12,13} We therefore investigated the influence of *H pylori* infection on the age related increase of pepsinogen A and pepsinogen C concentrations in healthy blood transfusion donors.

Methods

SUBJECTS

We examined sera obtained from 122 male and 69 female blood transfusion donors. The maximum age of these blood transfusion donors was 59 years. The age distribution of these donors is shown in Table I. IgG and IgA serum antibodies against *H pylori* and the serum concentrations of pepsinogen A and C were determined.

ANTI-H PYLORI ANTIBODIES

Specific IgA and IgG antibodies against *H pylori* were measured by a modified ELISA technique for IgA and IgG using conjugates labelled with immunoperoxidase specific for human IgA and IgG. The results were expressed as the absorbance index (AI):

$$\text{AI} = \frac{\text{mean OD reading (n=2) of patient's serum} - \text{mean OD of blank reading}}{\text{mean OD reading (n=2) of reference serum} - \text{mean OD of blank reading}}$$

where OD is the optical density. The procedure of the assay and determination of intra- and interassay variability of the ELISA technique has been described in detail by Peña *et al.*¹⁴

TABLE I Analysis of variance of the serum pepsinogen A concentrations in blood transfusion donors with and without serological evidence of *H pylori* infection stratified according to age

Age categories (yr)	All donors	<i>H pylori</i> (Negative)	<i>H pylori</i> (Positive)
20-29	49 (18) [58]	47 (6) [40]	52 (23) [18]
30-39	61 (29) [60]	53 (21) [47]	89 (38) [13]
40-49	63 (27) [52]	54 (20) [27]	73 (30) [25]
50-59	73 (34) [21]	55 (13) [11]	93 (41) [10]
All ages	59 (27) [191]	52 (19) [125]	73 (35) [66]*
F test on age	5.49	1.21	4.94
p value	<0.01	NS	<0.01

* F test on presence of *H pylori* = 30.70 (p value = < 0.01); Mean (SD) [no] of serum pepsinogen A concentrations ($\mu\text{g/l}$)

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Serum with an AI>0.32 for IgG anti-*H. pylori* was considered evidence of *H. pylori* infection.

PEPSINOGEN A AND PEPSINOGEN C

Serum pepsinogen A and C concentrations were determined by specific and sensitive radio-

immunoassays. Both techniques were developed in our department and have been validated in previous studies.⁶

The intraassay coefficients of variation varied from 3.0% to 10.2% at serum concentrations in the normal range for pepsinogen A and from 12.6 to 14.7% serum levels in the normal range for

TABLE II Analysis of variance of the serum pepsinogen C concentrations in blood transfusion donors with and without serological evidence of *H. pylori* infection stratified according to age

Age categories (yr)	All donors	<i>H. pylori</i> (Negative)	<i>H. pylori</i> (Positive)
20-29	11 (7) [58]	9 (5) [40]	15 (8) [18]
30-39	15 (13) [60]	11 (9) [47]	29 (16) [13]
40-49	17 (11) [52]	10 (5) [27]	25 (11) [25]
50-59	19 (16) [21]	8 (3) [11]	31 (16) [10]
All ages	15 (11) [191]	10 (7) [125]	24 (13) [66]*
F test on age	3.66	1.01	4.82
p value	0.01	NS	<0.01

* F test on presence of *H. pylori*=92.82 (p value=<<0.01); Mean (SD) [no] of serum pepsinogen C concentrations (µg/l)

TABLE III Analysis of variance of the serum pepsinogen A:C ratios in blood transfusion donors with and without serological evidence of *H. pylori* infection stratified according to age

Age categories (yr)	All donors	<i>H. pylori</i> (Negative)	<i>H. pylori</i> (Positive)
20-29	5.6 (4.1) [58]	6.4 (4.6) [40]	4.1 (1.9) [18]
30-39	5.1 (2.2) [60]	5.5 (2.2) [47]	3.4 (1.1) [13]
40-49	5.0 (3.1) [52]	6.7 (3.4) [27]	3.1 (1.2) [25]
50-59	5.2 (2.4) [21]	7.0 (1.9) [11]	3.2 (0.9) [10]
All ages	5.2 (3.1) [191]	6.2 (3.4) [125]	3.2 (1.4) [66]*
F test on age	0.58	1.11	1.65
p value	NS	NS	NS

* F test on presence of *H. pylori*=39.05 (p value=<<0.01); Mean (SD) [no] of serum pepsinogen A:C ratios

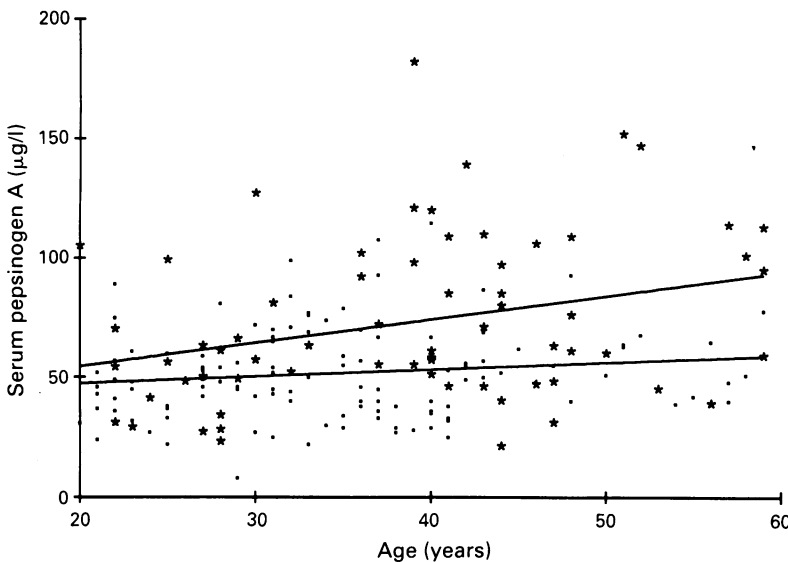


Figure 1: Relationship between serum pepsinogen A and age in *H. pylori* positive (*) and *H. pylori* negative (●) blood transfusion donors. Upper line: regression line ($r=0.30$; $p=0.01$) in *H. pylori* positive blood transfusion donors. Lower line: regression line ($r=0.15$; $p=0.10$) in *H. pylori* negative blood transfusion donors.

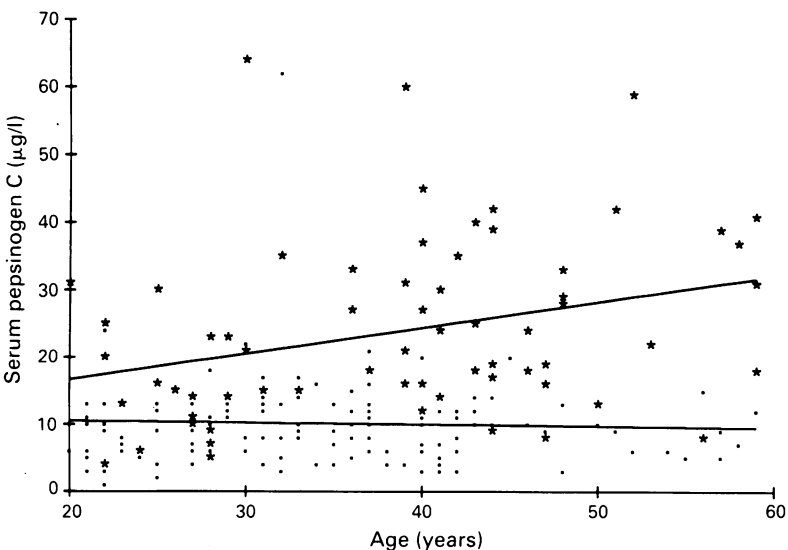


Figure 2: Relationship between serum pepsinogen C and age in *H. pylori* positive (*) and *H. pylori* negative (●) blood transfusion donors. Upper line: regression line ($r=0.31$; $p=0.01$) in *H. pylori* positive blood transfusion donors. Lower line: regression line ($r=-0.04$; $p=0.69$) in *H. pylori* negative blood transfusion donors.

pepsinogen C. All samples were measured in duplicate in the same assay. At all time intervals serum pepsinogen A:C ratios were calculated.

STATISTICAL ANALYSIS

Analysis of variance was used to compare the test results in the different age categories and with respect to *H. pylori* infection. The association between age and different test results was investigated by linear regression analysis and graphically displayed.

Results

In this study 191 healthy blood transfusion donors were studied, 70 donors had serological evidence of *H. pylori* infection.

Mean serum pepsinogen A and C concentrations in *H. pylori* positive blood transfusion donors were significantly ($p<<0.01$) higher than in *H. pylori* negative donors (Tables I, II). Analysis of variance showed that pepsinogen A and C concentrations differed significantly in the different age categories when we considered all blood transfusion donors (pepsinogen A: $F=5.49$, $p<0.01$, pepsinogen C: $F=3.66$, $p=0.01$) and *H. pylori* positive blood transfusion donors (pepsinogen A: $F=4.94$, $p<0.01$, pepsinogen C: $F=4.82$, $p<0.01$), the mean pepsinogen A and C concentrations being the highest in the older age categories. In *H. pylori* negative blood transfusion donors no difference of pepsinogen A and C concentrations could be shown in the different age categories (pepsinogen A: $F=1.21$, $p=ns$, pepsinogen C: $F=1.01$, $p=ns$). Serum pepsinogen C showed a relatively greater increase in than pepsinogen A in *H. pylori* positive transfusion donors. The above described non-parallel changes in serum pepsinogen A and C concentrations resulted in a significant ($p<<0.01$) lower mean serum pepsinogen A:C ration in *H. pylori* positive blood transfusion donors (Table III). Analysis of variance failed to show significant changes of pepsinogen A:C ratio in the different age categories in all blood transfusion donors regardless of their *H. pylori* status (Table III).

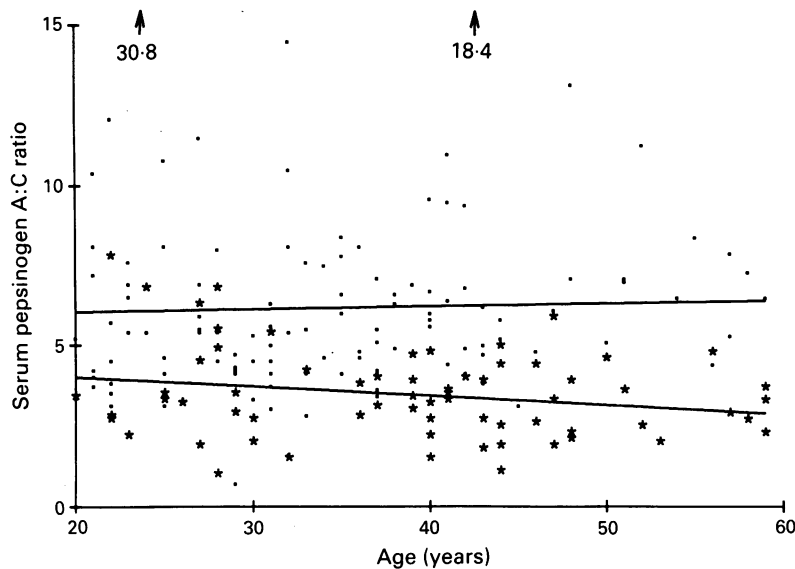


Figure 3: Relationship between serum pepsinogen A:C ratio and age in *H pylori* positive (*) and *H pylori* negative (●) blood transfusion donors. Upper line: regression line ($r=0.03$; $p=0.76$) in *H pylori* negative blood transfusion donors. Lower line: regression line ($r=-0.21$; $p=0.09$) in *H pylori* positive blood transfusion donors.

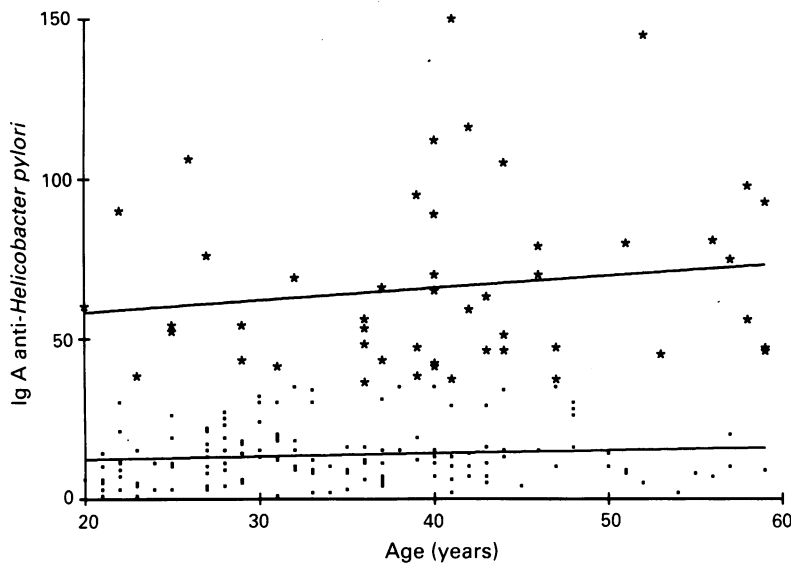


Figure 4: Relationship between the IgA absorbance index and age in *H pylori* positive (*) and *H pylori* negative (●) blood transfusion donors. Upper line: regression line ($r=0.27$; $p=0.03$) in *H pylori* positive blood transfusion donors. Lower line: regression line ($r=0.16$; $p=0.07$) in *H pylori* negative blood transfusion donors.

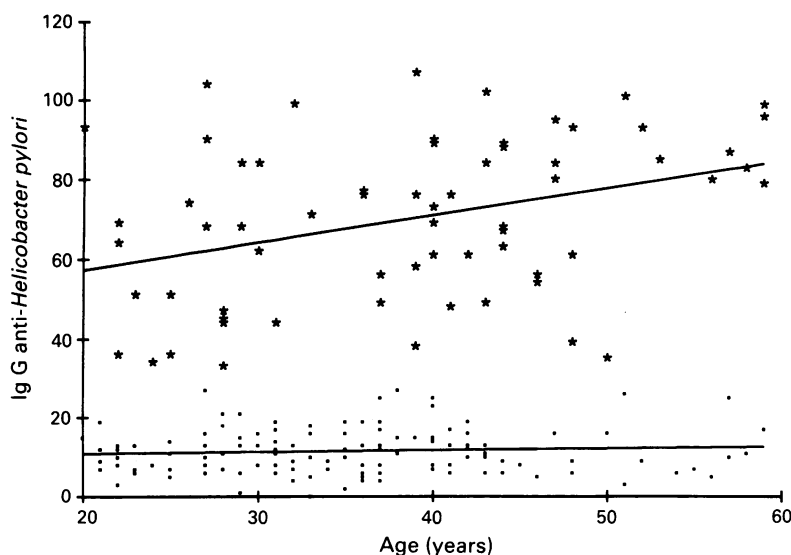


Figure 5: Relationship between the IgG absorbance index and age in *H pylori* positive (*) and *H pylori* negative (●) blood transfusion donors. Upper line: regression line ($r=0.36$; $p=0.03$) in *H pylori* positive blood transfusion donors. Lower line: regression line ($r=0.07$; $p=0.42$) in *H pylori* negative blood transfusion donors.

Serum pepsinogen A and C concentrations in *H pylori* positive and *H pylori* negative donors were plotted against age (Figs 1, 2) showing no correlation between age and serum pepsinogen A ($r=0.15$; $p=0.10$) or serum pepsinogen C ($r=-0.04$; $p=0.69$) in *H pylori* negative donors. In *H pylori* positive blood transfusion donors a weak but significant correlation between age and both serum pepsinogen A ($r=0.30$, $p=0.01$, Figure 1) and serum pepsinogen C ($r=0.31$, $p=0.01$, Figure 2) was found. No correlation between age and pepsinogen A:C ratio ($r=-0.21$, $p=0.09$, Figure 3) could be found in *H pylori* positive blood transfusion donors, nor in *H pylori* negative blood transfusion donors.

The IgA and IgG absorbance index in *H pylori* positive and *H pylori* negative donors were also plotted against age showing no correlation between age and the IgA ($r=0.16$, $p=0.07$, Figure 4) or the IgG ($r=0.07$, $p=0.42$, Figure 5) absorbance index in *H pylori* negative donors. In *H pylori* positive blood transfusion donors a weak but significant correlation between age and both the IgA ($r=0.27$, $p=0.03$, Figure 4) and the IgG ($r=0.35$, $p=0.03$, Figure 5) absorbance index was found.

Discussion

Serum pepsinogen A and C concentrations in an apparently healthy population increase with advancing age.⁵⁻⁷ The non-parallel changes in serum pepsinogen A and C also cause a slight decrease in the serum pepsinogen A:C ratio.^{6,7} As possible explanations for these phenomena both increasing pyloric gland metaplasia in the stomach⁵ and loss of renal function with advancing age¹⁵⁻¹⁷ have been put forward.

Our findings indicate that in a healthy population consisting of blood transfusion donors significant differences in serum pepsinogen A and C exist between *H pylori* positive and *H pylori* negative subjects (Tables I, II). Infection with *H pylori* is the cause of chronic superficial gastritis in which rises in pepsinogen A and C and a decrease in pepsinogen A:C ratio have been described.^{3,12,13} Treatment of *H pylori* infection leads to an improvement of gastritis¹⁸ and also causes a decrease in pepsinogen A¹³ and C^{19,20} with an increase in the pepsinogen A:C ratio. As the incidence of *H pylori* and its associated chronic superficial gastritis^{21,22} is higher in older age groups we suggest that the age dependent increase in serum pepsinogen A and C in a healthy control population is mainly caused by *H pylori* infection leading to chronic superficial gastritis. This point of view is supported by the fact that in *H pylori* negative blood transfusion donors no age related increase in serum pepsinogen concentrations could be found (Figs 1, 2). The slight but significant age related increase in serum pepsinogen A and C which we found in *H pylori* positive blood transfusion donors can possibly be explained by assuming that after triggering of chronic superficial gastritis by *H pylori* further progression of the gastritis occurs.^{23,24} In younger age groups chronic gastritis with minimal inflammatory infiltrate occurs in *H pylori* positive patients.²³ Longterm

biopsy follow up studies of Finnish and Estonian outpatient and population samples have shown an age related increase of severity in chronic gastritis caused by *H pylori*.²⁵ Release of pepsinogen A and C into the circulation in response to this inflammation²⁶ is probably the cause of the observed age related increase of pepsinogen A and C in *H pylori* positive blood transfusion donors.

The cause of our finding that the IgA and IgG absorbance index show an increase with advancing age is unclear but might also reflect progression of chronic superficial gastritis with advancing age. Our findings suggest that the rise in serum pepsinogen A which has been described in relatives of duodenal ulcer patients could be at least partly explained by *H pylori* infection which is also common in members of the same family.²⁷⁻²⁹ In these subjects a genetic predisposition to duodenal ulcer disease has been suggested^{30,31} in which *H pylori* may be the environmental factor which converts genetic predisposition into disease. We therefore conclude that in healthy blood transfusion donors no age related increase in serum pepsinogen A and C can be shown unless there is an infection with *H pylori*. Serum pepsinogen A and C concentrations should be related therefore to the presence or absence of *H pylori* infection.

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