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Tamoxifen exposure in relation to gastric adenocarcinoma development

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ABSTRACT

Epidemiological research has indicated that the anti-oestrogen tamoxifen, used in breast cancer therapy, may increase the risk of gastric adenocarcinoma of the intestinal but not of the diffuse type. To test this hypothesis, and evaluate possible involvement of oestrogen receptors (ERs), we conducted a study amongst tamoxifen users and non-users. The study participants comprised women in the county of Stockholm who in the Swedish Cancer Register were first recorded with breast cancer and subsequently gastric cancer during the period January 1958–August 2005. Medical records were scrutinised to verify the diagnoses and classify into use or non-use of tamoxifen. Tumour material was reviewed histologically to verify gastric adenocarcinoma diagnosis and classify these cancers into intestinal or diffuse type. Intestinal adenocarcinomas were analysed immunohistochemically for the presence of ER alpha, beta and beta cx. Amongst 68 women with verified gastric adenocarcinoma, 30 had been treated with tamoxifen and 38 not. The intestinal type of gastric adenocarcinoma was not more frequent amongst tamoxifen users (27%) than amongst non-users (34%) ($p = 0.601$). There were no material differences between the tamoxifen groups regarding distribution of any of the three ERs of the intestinal adenocarcinoma specimens. Tamoxifen users had a shorter latency between breast cancer and gastric adenocarcinoma (4 versus 13 years) which was similar in the intestinal and diffuse types. This study does not support the hypothesis that tamoxifen increases the isolated risk of the intestinal type, but it indicates that tamoxifen use might accelerate the tumour progression or increase the overall risk of gastric adenocarcinoma.

1. Introduction

There is a need to clarify the potential influence of oestrogen and anti-oestrogen on the risk of developing gastric cancer. Epidemiological studies have provided some support in favour of the hypothesis that the male predominance in the incidence of gastric adenocarcinoma might be due to sex hormonal exposures, mainly through a decreased risk amongst women who are highly exposed to oestrogens.[1–5] The male predominance (overall sex ratio 2–3:1)[6] might be limited to the intestinal

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histological type of gastric adenocarcinoma, since no such sex difference has been found for the diffuse type.[7] This male to female ratio increases up to the age of about 60 years, after which it decreases, suggesting that endogenous oestrogens may protect women.[7] Amongst studies of exogenous oestrogen exposure in relation to risk of gastric adenocarcinoma, there have been reports of a decreased risk amongst women using hormone replacement therapy,[2] and amongst men using oestrogen therapy for prostate cancer,[3] and an increased risk amongst women using the anti-oestrogen tamoxifen for breast cancer.[1,8–11] The biological mechanism by which oestrogen or anti-oestrogen may be involved in the above findings is not established. On the basis of the available literature, we hypothesised that tamoxifen increases the risk only of the intestinal type of gastric adenocarcinoma, and not of the diffuse type. If this is true, gastric adenocarcinomas occurring amongst tamoxifen users should more often be of the intestinal than of the diffuse type, compared to the gastric adenocarcinomas occurring amongst non-users of tamoxifen. Furthermore, we hypothesised that any anti-oestrogen effect is mediated by oestrogen receptors (ERs). There are two types of ER, alpha (ERα)[12] and beta (ERβ),[13] both of which have been identified in gastric mucosa.[14,15] Moreover, a splicing variant of ERβ, namely ERβcx, has been discovered[16] and we have recently found this type of ER in gastric tissue (data not shown). We tested these hypotheses by studies on a population-based collection of gastric adenocarcinoma specimens amongst patients with a known tamoxifen exposure status. We compared users and non-users of tamoxifen regarding (1) the distribution of the intestinal and diffuse types of gastric adenocarcinoma and (2) the expression of ERs in the intestinal type of gastric adenocarcinoma.

2. Materials and methods

2.1. *Identification of patients with breast cancer and a subsequent gastric cancer*

The Swedish Cancer Register contains data that enabled complete identification of patients with a breast cancer diagnosis who subsequently developed gastric cancer, both occurring during the study period January 1958–August 2005 in the county of Stockholm. Since 1958 all clinicians and pathologists in Sweden have been required to report all cancer cases to the Swedish Cancer Register, a register which has been found to have high validity and at least 98% completeness with regard to gastric cancer registration.[17] For the registration of cancer diagnoses, the Register has used the 7th revision of the International Classification of Diseases (ICD) (WHO/HS/CANC/24.1 Code for Anatomical Location) between 1958 and 1986. For the years 1987–1992, the ICD-9 coding system has been followed (WHO 9th revision 1976), for the period 1993–2004 the ICD-O-2/ICD-10 (International Classification of Diseases for Oncology. Second Edition. WHO Geneva 1990) and since 2005 the ICD-O/3 (3rd Edition. WHO Geneva 2000) is being used. The Swedish Cancer Register translates the different classifications to ICD-7 for research purposes. Through the Register, we identified 211 female patients with a recorded breast cancer diagnosis and a subsequent gastric cancer in the county of Stockholm during the study period.

2.2. *Evaluation of cancer diagnoses and tamoxifen exposure*

Amongst the 211 primarily identified patients we were able to collect valid medical records of 107 (51%). These records were reviewed to verify the diagnoses and their dates, and to classify each patient with regard to tamoxifen treatment status (yes or no). We obtained data that verified the diagnoses and dates as well as tamoxifen exposure in 101 patients (94%). Of these, 8 were excluded since their gastric cancer was diagnosed within 1 year of the breast cancer diagnosis. This exclusion was made in order to avoid surveillance bias, which would imply that patients with a newly diagnosed breast cancer would be more likely to have another cancer detected because of the ongoing diagnostic examinations.

2.3. *Evaluation of gastric adenocarcinoma type*

Of the remaining 93 patients, we obtained histological specimens from 76 (82%). Two investigators (EC and CAR), who were kept blinded with regard to tamoxifen exposure, confirmed or refuted the gastric adenocarcinoma diagnosis by reviewing histological specimens fixed with haematoxylin. Eight gastric tumours were excluded since a gastric adenocarcinoma could not be verified, leaving 68 patients with gastric adenocarcinoma for the final study cohort. These patients were categorised according to Laurén's classification into the intestinal or diffuse type.[18] Tumours with a combination of these two histological types were classified as being of the diffuse type, as proposed by Ming.[19]

2.4. *Evaluation of oestrogen receptors*

Amongst the 21 remaining patients with gastric adenocarcinoma of the intestinal type, only a limited amount of tissue was available in five cases, leaving 16 cases for final ER analysis. Paraffin-embedded material was collected and sections for immunohistochemistry were cut. Three types of ER were investigated, ERα, β and βcx. One investigator (EC) performed the immunohistochemistry according to a previously described protocol.[20] In brief, we used the following antibodies and dilutions: rabbit anti-ERα from Santa Cruz Biotechnology (1:120), chicken polyclonal antibody anti-ERβ 503 IgY (1:200) and anti-ERβcx sheep anticlonal antibody (1:200), the last two produced at our laboratory. Human breast tissue was obtained for positive control. Secondary antibodies were applied, the slides were incubated with avidin biotin complex (ABC) (Vector lab) and coloured with 3,3′-diaminobenzidine tetrahydrochloride substrate (DAKO). Finally, they were counterstained with Mayer's haematoxylin. Immunohistochemical results were evaluated according to the Wang method[21] by two investigators (EC and CAR) who were blinded to the tamoxifen exposure. Following the recommendation of Wang,[21] selection of one field is better than when the results are based on an average count of three or five fields and therefore areas with the highest degree of ER expression were evaluated. Specimens with 50% or more of the cells, in a high power field, positive for ERs were considered positive. ERs are usually found in the nucleus,[22] but

Table 1 – Age at onset of breast cancer and gastric adenocarcinoma amongst women diagnosed with both these tumours in the county of Stockholm between 1958 and 2005

	Tamoxifen	Non-tamoxifen	Total
Number of patients (%)	30 (44)	38 (56)	68 (100)
Mean age at breast cancer diagnosis in years (range)	67 (42–87)	66 (46–80)	67 (42–87)
Mean age at gastric adenocarcinoma diagnosis in years (range)	72 (47–89)	79 (59–94)	76 (47–94)
Mean number of years between breast and gastric adenocarcinoma diagnosis in years (range)	All: 4 (2–9) Intestinal: 5 (2–7) Diffuse: 4 (2–9)	All: 13 (1–35) Intestinal: 12 (1–35) Diffuse: 13 (4–33)	All: 9 (1–35) Intestinal: 9 (1–35) Diffuse: 9 (2–33)

The tamoxifen exposure was categorised into use or non-use.

immunoreactivity in the cytoplasm has also been shown.[23] In this study, sections with positive immunoreactivity in the nucleus were therefore classified as a nucleus-positive group, whilst those with exclusively cytoplasmic expression were assigned to the non-nuclear-staining group. We examined both the gastric cancer tissue and the non-tumour tissue adjacent to the tumour.

2.5. *Statistical analysis*

Logistic regression was utilised to estimate *p*-values. Fisher's exact test was also used to compare the frequency distributions of the three types of ER amongst the two groups of patients (data not shown). All statistical analyses were performed with STATA version 9.2 (Stata Corporation, College Station, TX, USA). All reported probabilities (*p*-values) were two-sided, and those less than 0.05 were considered statistically significant.

Table 2 – Histological types of gastric adenocarcinoma according to Laurén classification in women with previous breast cancer diagnosed in the county of Stockholm between 1958 and 2005

Tamoxifen treatment	Histology			
	Intestinal number (%)	Diffuse number (%)	Total number (%)	*p*-Value
No	13 (34)	25 (66)	38	Reference
Yes	8 (27)	22 (73)	30	0.60
Total	21 (31)	47 (69)	68	

The tamoxifen exposure was categorised into use or non-use.

2.6. *Ethics*

The regional ethics committee at Karolinska Institutet in Stockholm, Sweden, approved this study.

3. Results

3.1. *Latency interval between breast cancer and gastric adenocarcinoma amongst patients with and without tamoxifen*

Table 1 shows the mean age at onset of the breast cancer and of the gastric adenocarcinoma amongst the 68 study patients with both these diagnoses. The mean age at breast cancer onset was similar in the groups with and without tamoxifen treatment ($p = 0.54$), whilst the age at onset of gastric adenocarcinoma was lower amongst patients treated with tamoxifen ($p < 0.05$). The latency interval between the first occurrence of these two tumours was on average almost 9 years shorter in the tamoxifen-treated women than in those without such treatment ($p < 0.05$). This difference in latency interval between users and non-users of tamoxifen was similar in stratified analyses of the intestinal and the diffuse type of gastric adenocarcinoma (Table 1). The difference in latency interval between users and non-users of tamoxifen remained after restriction to patients who received a breast cancer diagnosis in 1978 or later (the year

Table 3 – Cancer tissue – nuclear staining

Tamoxifen treatment	Total number	ERα+ number (%) *p*-Value	ERβ+ number (%) *p*-Value	ERβcx+ number (%) *p*-Value
No	9 (8 cases stained for ERα)	4 (50) Reference	0 (0) Reference	0 (0) Reference
Yes	7	1 (14) 0.16	0 (0)	1 (14) –
Total	16	5 (33)	0	1 (6)

Cases of intestinal gastric adenocarcinoma, following previous breast cancer, investigated immunohistochemically regarding the presence of oestrogen receptors (ER) alpha (α), beta (β) and beta cx (βcx). Positive cases (+) were defined as those with positively stained nuclei in the gastric adenocarcinoma tissue. The tamoxifen exposure was categorised into use or non-use.

Table 4 – Cancer tissue – non-nuclear staining

Tamoxifen treatment	Total number	ERα+ number (%) *p*-Value	ERβ+ number (%) *p*-Value	ERβcx+ number (%) *p*-Value
No	9 (8 cases stained for ERα)	1 (13) Reference	0 (0) Reference	3 (33) Reference
Yes	7	3 (43) 0.2	1 (14) –	1 (14) 0.39
Total	16	4 (27)	1 (6)	4 (25)

Cases of intestinal gastric adenocarcinoma, following previous breast cancer, investigated immunohistochemically regarding the presence of oestrogen receptors (ER) alpha (α), beta (β) and beta cx (βcx). Positive cases (+) were defined as those with positive staining in intracellular granules and/or cytoplasm, but no staining in the nuclei in the gastric adenocarcinoma tissue. The tamoxifen exposure was categorised into use or non-use.

in which the first tamoxifen treatment was registered in our cohort) (Table 7). Breast cancer patients who had been exposed to tamoxifen were affected by gastric cancer in mean after 4 years compared to 10 years amongst unexposed patients, again without differences between the two histological types.

3.2. *Distribution of intestinal and diffuse types of gastric adenocarcinoma amongst patients with and without tamoxifen*

The distribution of the two histological types of gastric adenocarcinoma in the tamoxifen users and non-users is presented

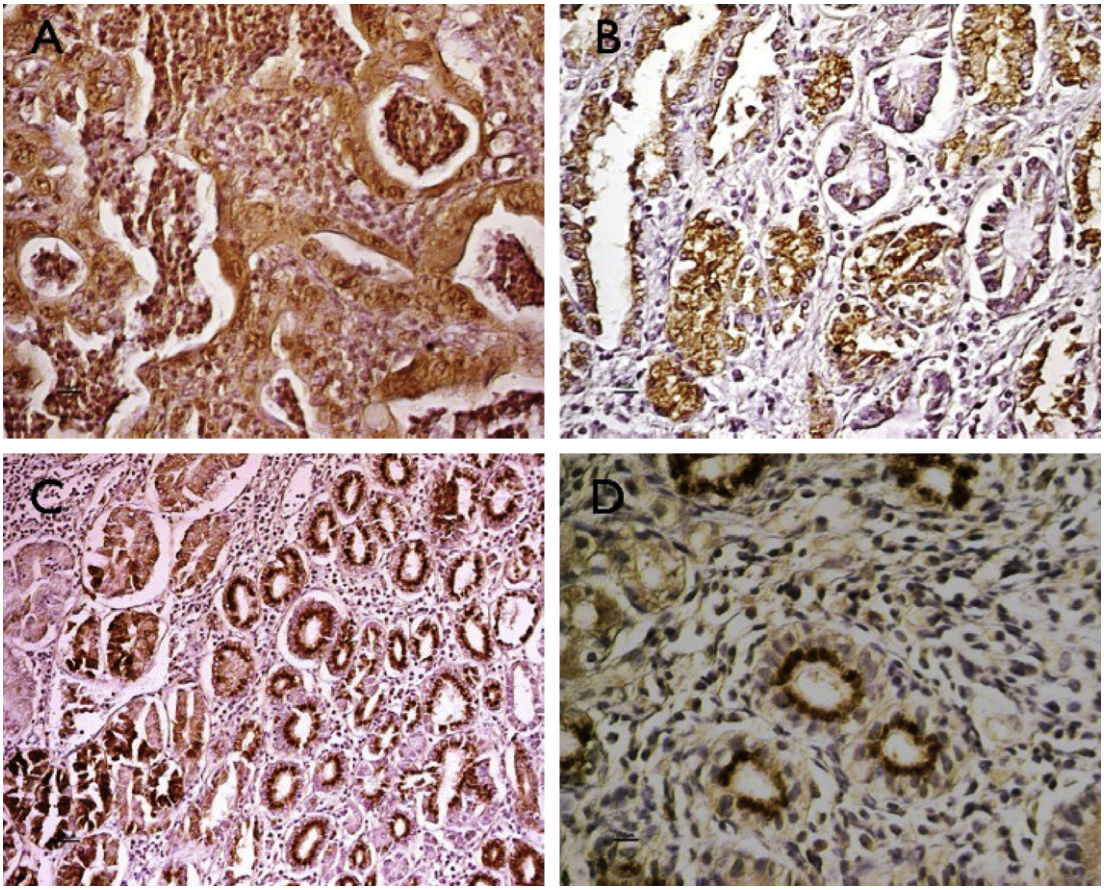

Fig. 1 – (A) Gastric adenocarcinoma of the intestinal type expressing nuclear oestrogen receptor alpha, in a tamoxifen-exposed woman. (B) Non-tumour pyloric glands adjacent to intestinal gastric adenocarcinoma (not shown), expressing oestrogen receptor beta in the cytoplasm, in a women with no previous tamoxifen treatment. (C) Non-tumour glands adjacent to intestinal gastric adenocarcinoma (not shown), expressing oestrogen receptor beta cx in supra-nuclear granules, in a woman with no previous tamoxifen treatment. (D) Non-tumour pyloric glands adjacent to intestinal gastric adenocarcinoma (not shown), expressing oestrogen receptor beta cx in supra-nuclear granules, in a woman with previous tamoxifen treatment.

Table 5 – Non-tumour tissue – nuclear staining

Tamoxifen treatment	Total number	ERα+ number (%) *p*-Value	ERβ+ number (%) *p*-Value	ERβcx+ number (%) *p*-Value
No	7 (6 cases stained for ERα)	2 (33)	1 (14)	1 (14)
		Reference	Reference	Reference
Yes	7	1 (14)	0 (0)	0 (0)
		0.42	–	–
Total	14	3 (23)	1 (7)	1 (7)

Cases of intestinal gastric adenocarcinoma, following previous breast cancer, investigated immunohistochemically regarding the presence of oestrogen receptors (ER) alpha (α), beta (β) and beta cx (βcx). Positive cases (+) were defined as those with positively stained nuclei in the non-tumour gastric mucosa (adjacent to the gastric adenocarcinoma). The tamoxifen exposure was categorised into use or non-use.

in Table 2. The intestinal type was not overrepresented in the tamoxifen group (27%) compared to the non-tamoxifen group (34%), and no statistically significant difference was identified ($p = 0.6$).

3.3. *Oestrogen receptors alpha, beta and beta cx in intestinal gastric adenocarcinoma tissue in patients with and without tamoxifen*

The occurrence of ERα-positive staining in the nucleus was seemingly higher in the non-tamoxifen-treated group than in the tamoxifen group, but the difference was statistically non-significant (Table 3). No ERβ cases were found, and only one case of ERβcx was noted (in the tamoxifen group). The non-nuclear staining suggested that ERα and ERβ were more common in the tamoxifen group, whilst ERβcx was more common in the non-tamoxifen group, but these results were not statistically significant (Table 4). An example of ERα expression in gastric adenocarcinoma is shown in Fig. 1A.

3.4. *Oestrogen receptors alpha, beta and beta cx in non-tumour gastric mucosa adjacent to intestinal gastric adenocarcinoma in patients with and without tamoxifen*

Table 5 shows, in cases of intestinal gastric adenocarcinoma, the distribution of the three ER types, by tamoxifen treatment, in the nuclear parts of the cell in the non-tumour gastric mucosa (adjacent to the gastric adenocarcinoma). In Table 6, corresponding results are given for the non-nuclear staining in non-tumour gastric mucosa. The results were similar to those of the adenocarcinoma tissue. In the nuclear staining, all ERs seemed more common in the non-tamoxifen group, whilst the opposite was found in the non-nuclear staining, but these results were without statistical significance. In general, there were indications of an increased ERβ positivity and a decreased ERα positivity in the non-tumour gastric mucosa compared to the cancer tissue (Tables 3–6). Examples of ERβ and ERβcx expression in non-tumour gastric mucosa are shown in Fig. 1B–D.

4. Discussion

This study did not provide any evidence of a selectively increased occurrence of the intestinal type of gastric adenocarcinoma amongst tamoxifen users. Nor did we find any obvious difference in the presence of ERs of the intestinal gastric adenocarcinomas amongst users and non-users of tamoxifen. The latency interval between the occurrence of breast cancer and gastric adenocarcinoma was shorter, however, amongst tamoxifen users than amongst non-users, irrespective of whether the gastric adenocarcinoma was of the intestinal or the diffuse type.

We aimed at population-based sampling without misclassification of tumours or exposure (tamoxifen use). To achieve this we collected original data from the virtually complete Swedish Cancer Register,[17] verified the tumours and use of tamoxifen through a scrutiny of medical records, and reviewed the histological specimens. A risk of selection bias was introduced, however, mainly by the limited rate of complete medical records and by the lack of some histological

Table 6 – Non-tumour tissue – non-nuclear staining

Tamoxifen treatment	Total number	ERα+ number (%) *p*-Value	ERβ+ number (%) *p*-Value	ERβcx+ number (%) *p*-Value
No	7 (6 cases stained for ERα)	2 (33)	4 (57)	4 (57)
		Reference	Reference	Reference
Yes	7	5 (71)	4 (57)	5 (71)
		0.18	1	0.57
Total	14	7 (54)	8 (57)	9 (64)

Cases of intestinal gastric adenocarcinoma, following previous breast cancer, investigated immunohistochemically regarding the presence of oestrogen receptors (ER) alpha (α), beta (β) and beta cx (βcx). Positive cases (+) were defined as those with positive staining in intracellular granules and/or cytoplasm, but no staining in the nuclei in the non-tumour gastric mucosa (adjacent to the gastric adenocarcinoma). The tamoxifen exposure was categorised into use or non-use.

specimens. Problems emerged from the fact that medical records had to be obtained from surgical and oncological departments, as well as private practitioners, spanning over a period of more than four decades (1958–2005). It is unlikely, however, that this incomplete availability of medical records or specimens would be linked with the tamoxifen exposure, and thus our study should not have been biased by differential misclassification. Differential misclassification of exposure and outcome was prohibited by our strict blinding of the examiners of the medical records, histology and ERs. A limitation of the study is the retrospective data collection, which did not enable us to adjust for potential confounding by risk factors for gastric adenocarcinoma, i.e. heredity, *Helicobacter pylori* infection, dietary factors, tobacco smoking, alcohol consumption and obesity.[24] The distribution of the intestinal and diffuse types of gastric adenocarcinoma is not, however, linked with these risk factors[25–29] and these factors should therefore, by definition, not act as confounders in our analyses of histological type. Another potential problem of the study is the exposure assessment. Although we had valid data regarding the use of tamoxifen, the duration and potential latency of a harmful effect of such use is uncertain. However, tamoxifen is typically used for long periods of time (about 5 years) with the purpose of decreasing the risk of recurrent breast cancer.[30] Moreover, patients who received the gastric cancer diagnosis within a year of the breast cancer onset were excluded in order to avoid surveillance bias. Although the data collection encompassed a period of four decades and covered the entire county of Stockholm, the occurrence of breast cancer followed by a gastric adenocarcinoma is rare, a fact which limited the statistical power, particularly regarding the ER analyses.

Refuting our hypothesis, our results did not reveal any increased frequency of the intestinal compared to the diffuse type of gastric adenocarcinoma amongst tamoxifen users, and no material difference in ER occurrence was found between the tamoxifen groups. Interestingly, however, the shorter latency interval between the onset of breast and gastric cancer amongst the tamoxifen-exposed women indicates that tamoxifen therapy might actually accelerate the development of gastric adenocarcinoma. This should, however, be interpreted with caution, as there is a risk for selection bias. Tamoxifen was introduced in the later half of the total follow-up period (1958–2005), and patients having that treatment who would have developed gastric cancer at a much later date (long latency interval) were not included in the analysis for the reason that the follow-up period in the study had ended. As a result, only those with a short latency interval would have been included in the analysis. However, stratified analysis showed that there was still a difference between the groups regarding the latency between these two cancers. The first breast cancer patient in our cohort who received tamoxifen treatment was registered in 1978. If only patients who were diagnosed with breast cancer in 1978 or later are analysed, the latency interval in the tamoxifen group is 4 years, whilst that in the non-tamoxifen group is 10 years ($p < 0.05$, analysis of variance) (Table 7). The latency interval seemed to be independent of the histological type.

A possible explanation for these findings is that tamoxifen increases the overall risk of gastric adenocarcinoma, and does not affect the risk of the intestinal type more than that of the diffuse, at least not in our study population. This interpretation is in line with the results of our previous cohort study of tamoxifen use as a risk factor for gastric cancer,[1] as well as with other studies that have indicated that tamoxifen treatment might increase the risk of gastric cancer.[8–11,32–36]

A mechanism that might explain the shorter latency interval between breast cancer and gastric adenocarcinoma in the tamoxifen users is that tamoxifen blocks a possible protective effect of endogenous oestrogen against the development of gastric adenocarcinoma. Women who receive tamoxifen may be the ones with high ERalpha in their breasts and they may therefore also have a higher level of ERalpha in their gastric mucosa, and thus be more vulnerable to blockage of ERs. Moreover, the difference in latency to diagnosis of gastric cancer might also be influenced by a more prevalent use of tamoxifen amongst women with more advanced breast cancer, and these women might be more vulnerable and therefore more prone to develop a second cancer. The mechanism by which oestrogen may protect against gastric adenocarcinoma is not yet established. Oestrogen affects the expression of trefoil factor (TTF) genes. TTF proteins protect mucous epithelia from a range of insults and contribute to mucosal repair.[32] The expression of these genes is reduced in precancerous conditions and in

Table 7 – Age at onset of breast cancer and gastric adenocarcinoma amongst women diagnosed with both these tumours in the county of Stockholm between 1978 and 2005

	Tamoxifen	Non-tamoxifen	Total	*p*-Value
Number of patients (%)	30 (68%)	14 (32%)	44 (100%)	
Mean age at breast cancer diagnosis in years (range) (p50)	67 (42–87) (67)	66 (56–79) (68)	67 (42–87) (67)	0.76
Mean age at gastric adenocarcinoma diagnosis in years (range) (p50)	72 (47–89) (72)	76 (65–88) (77)	73 (47–89) (73)	0.11
Mean number of years between breast and gastric adenocarcinoma diagnosis in years (range) (p50)	4 (2–9) (4)	10 (4–19) (10)	6 (2–19) (6)	0.0

The tamoxifen exposure was categorised into use or non-use.

gastric cancer,[33] and oestrogen has been found to stimulate their expression.[34] Others suggest that oestrogen may bind to ERs and inhibit the expression of c-erbB-2 oncogene or the expression of p185.[35] The latter is associated with the progression of gastric cancer.[35] Tamoxifen may act by inhibiting these protective actions of oestrogens. Moreover, tamoxifen has been found to regulate expression of transforming growth factor-α and β (TGF), and to bind to calcium channels and protein kinase C,[36] but it is not known through which of these mechanisms, if any, it acts on the gastric mucosa. The suggested actions of tamoxifen in gastric mucosa – if indeed true – may explain why there was a shorter latency interval between the breast and gastric cancer diagnoses irrespective of the histological type of gastric adenocarcinoma. However, more studies are need to establish the mechanism behind our finding. For example the expression of various proteins (e.g. TTF, TGF-α and β) should be measured in patients with gastric cancer after tamoxifen treatment and compared to those with no such exposure as well to that of normal gastric mucosa.

This study alone cannot provide evidence for any changes in clinical praxis, but if our results regarding the shorter time latency is confirmed by others, tamoxifen treatment should perhaps not be used in young women with a strong heredity for gastric cancer. It may be also interesting to study if tamoxifen exposure affects the latency interval between breast cancer and oesophageal adenocarcinoma which also shows a male dominance in its incidence.[6]

In conclusion, this study did not provide any evidence in favour of the hypothesis of an isolated increase in the risk of the intestinal type, and not of the diffuse type, of gastric adenocarcinoma amongst users of tamoxifen. The shorter latency interval between breast cancer and gastric adenocarcinoma, irrespective of the histological type, amongst tamoxifen users, however, indicates that tamoxifen might speed up the progression of or is a risk factor for overall gastric adenocarcinoma.

Conflict of interest statement

Professor Jan-Åke Gustafsson is a consultant, shareholder and grant receiver from Karo Bio, a campus based Biotech Company.

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