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Immunohistochemical Localisation of Hydroxysteroid Sulphotransferase in Human Breast Carcinoma Tissue: a Preliminary Study

S. Sharp, J.M. Anderson and M.W.H. Coughtrie

Understanding the function and regulation of the metabolism of steroid hormones by breast tumours will be instrumental to the development of novel treatments for this widespread disease. We have examined the expression of hydroxysteroid sulphotransferase, an enzyme which inactivates many steroids, in particular androgens, in normal breast tissue and in six ductal-type mammary carcinomas using immunohistochemistry. The enzyme is not expressed in the epithelial cells which line the normal breast duct, but is present in significant amounts in neoplastic cells, suggesting that the gene encoding this protein is activated at some stage of the neoplastic transformation. The implications of this finding for the role of steroid metabolism in breast cancer are discussed.

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INTRODUCTION

EFFECTIVE TREATMENT of hormone-dependent breast carcinomas in their early stages is dependent on the response to hormonal manipulation, and the hormonal micro-environment within breast tumour cells is an important factor in determining this response [1]. Exposure of breast carcinoma cells to free oestrogens has a significant effect on tumour growth rate, and such exposure may be limited by the conjugation (and resultant inactivation) through sulphation of both oestrogens and their androgenic precursors by the sulphotransferase enzyme system.

Reversible sulphation, catalysed by sulphotransferases (STs) and sulphatases, is becoming increasingly recognised as a potent and specific means of regulating steroid hormone activity in target tissues [2, 3], since the sulphates are biologically inactive and more water soluble than the parent compounds.

Quantitatively, the most important steroid conjugates present in the female circulation are oestrone sulphate (E_1S) and dehydroepiandrosterone sulphate (DHEA-S). Physiological concentrations of these substrates have been demonstrated to be utilised by MCF-7 human breast cancer cells [4, 5] and also human breast cancer tissue [6], in which they are cleaved by E_1 sulphatase or DHEA sulphatase enzymes to release the free steroids. These substrates may then be further metabolised to biologically-active steroids.

Conjugation of both free oestrogenic and androgenic steroids to form their sulphates has also been shown to occur within breast cancer cell lines. These reactions are catalysed by the ST enzyme family, of which two isoforms, hydroxysteroid-ST (HST, EC 2.8:2.2) and an oestrogen-ST (EST, EC 2.8:2.4) are present in human mammary cancer cell lines [7, 8]. However, the regulation of expression of these enzymes has, as yet, not been clearly defined, and their location within breast tissue and precise role in hormonal control within these cells is still unknown.

The present study was undertaken to investigate the location of the HST present in human breast carcinoma tissue. Recent work in this laboratory has led to the production of an antibody directed against rat liver HST [9], which has been shown to recognise the human HST on immunoblot [9] and immunohistochemical [10] analyses. We used this antibody to localise this enzyme by immunostaining of formalin-fixed, paraffin wax-embedded breast tissue sections from a number of confirmed breast carcinoma patients. Preliminary findings demonstrated, in all cases, that the enzyme was present throughout the cytoplasm in carcinomatous ductal epithelial cells, whereas normal ductal epithelial cells were unstained.

MATERIALS AND METHODS

Chemicals

Avidin-biotin blocking solutions were obtained from Vector Laboratories (Peterborough, U.K.). Biotinylated goat anti-rabbit IgG and streptavidin–peroxidase complex were obtained from Biogenex (Croydon, U.K.) and steroid receptor immunological assay kits were obtained from Abbott Laboratories (Chicago, U.S.A.). All other reagents were of analytical grade and purchased from commonly used suppliers.

Tissue preparation

Blocks of human breast carcinoma samples were obtained from the archives of the Department of Pathology, Ninewells Hospital and Medical School. Specimens were examined from 6 patients who had undergone excisional biopsy or mastectomy. The tumours were classified as grade 2 or 3 ductal carcinomas. Tissues were immediately formalin-fixed and paraffin-embedded according to the method of Hopwood and colleagues [11]. Sections (10 μm) were mounted on slides and heated at 37°C overnight.

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Immunohistochemical staining

Anti-(rat liver HST), which specifically recognises equivalent epitopes on the human ortholog [9, 10], was used to probe sections of breast tumour tissue. Paraffin was removed from the specimens with Histoclear, rehydrated in ethanol and rinsed in water. Endogenous peroxidase activity was inactivated by incubation for 5 min with 7.5% H_2O_2, and blocking of avidin–biotin binding sites was carried out using the kit provided by Vector Laboratories. After a 5-min incubation period in 20% normal human serum, sections were incubated overnight at 4°C in primary antibody (IgG fraction) at concentrations of 1 or 2 μg/ml, and control sections were exposed to pre-immune rabbit IgG at the same concentrations. After washing with phosphate-buffered saline (PBS), samples were incubated at room temperature in biotinylated goat anti-(rabbit IgG), diluted 1/25 for 20 min, washed in PBS and then incubated in streptavidin–peroxidase complex for 20 min. They were then rinsed with PBS, and the peroxidase reaction visualised with 0.01% diaminobenzidine and 0.003% H_2O_2 for 5 min. Sections were counterstained with Mayer's haematoxylin reagent.

Receptor analyses

Segments of tumour tissue were obtained from biopsy or mastectomy samples immediately upon removal at surgery, and cytosol samples were prepared and assayed for the presence of oestrogen and progesterone receptors by the Department of Surgery, Ninewells Hospital and Medical School according to the procedure described by the manufacturer (Abbott Laboratories).

Positive control immunohistochemistry

Human liver biopsy material (histologically normal) was used to demonstrate the immunohistochemical staining of HST in another adult human tissue, and was processed in exactly the same way as for breast tumour tissue. Previous work from this laboratory has documented the use of this antibody for the immunohistochemical detection of HST in rat and fetal human tissues [9, 10].

RESULTS

A series of six breast carcinoma patients were examined, 3 of whom were positive for either oestrogen or progesterone receptors or both, and 3 of whom were negative for both receptor types. Figure 1 shows results from two cases, which were representative of data obtained on all six samples. In all cases examined, carcinomatous epithelium showed positive immunostaining with the antibody to human HST (Figures 1a,b). The staining reaction product was finely granular and dispersed evenly throughout the cytoplasm of the carcinomatous epithelium without highlighting surface membranes. Nuclei were unstained. Some variation in intensity of staining was seen in different tumours, but both well-differentiated adenocarcinomas and poorly-differentiated tumours were distinctly immunopositive. Stromal components of neoplasms attracted minimal immunostaining and were judged to be negative. No obvious differences were seen between tumours which were positive or negative for the oestrogen and progesterone receptors.

Some components of normal breast tissue were also immunostained with the anti-(HST) antibody preparation. Very light immunostaining was seen over secretory epithelium and the myoepithelium of the terminal duct lobular units (Figure 1c). By contrast, the lining epithelium of more distal ducts was invariably negative (Figure 1d). Occasionally, positively-stain-

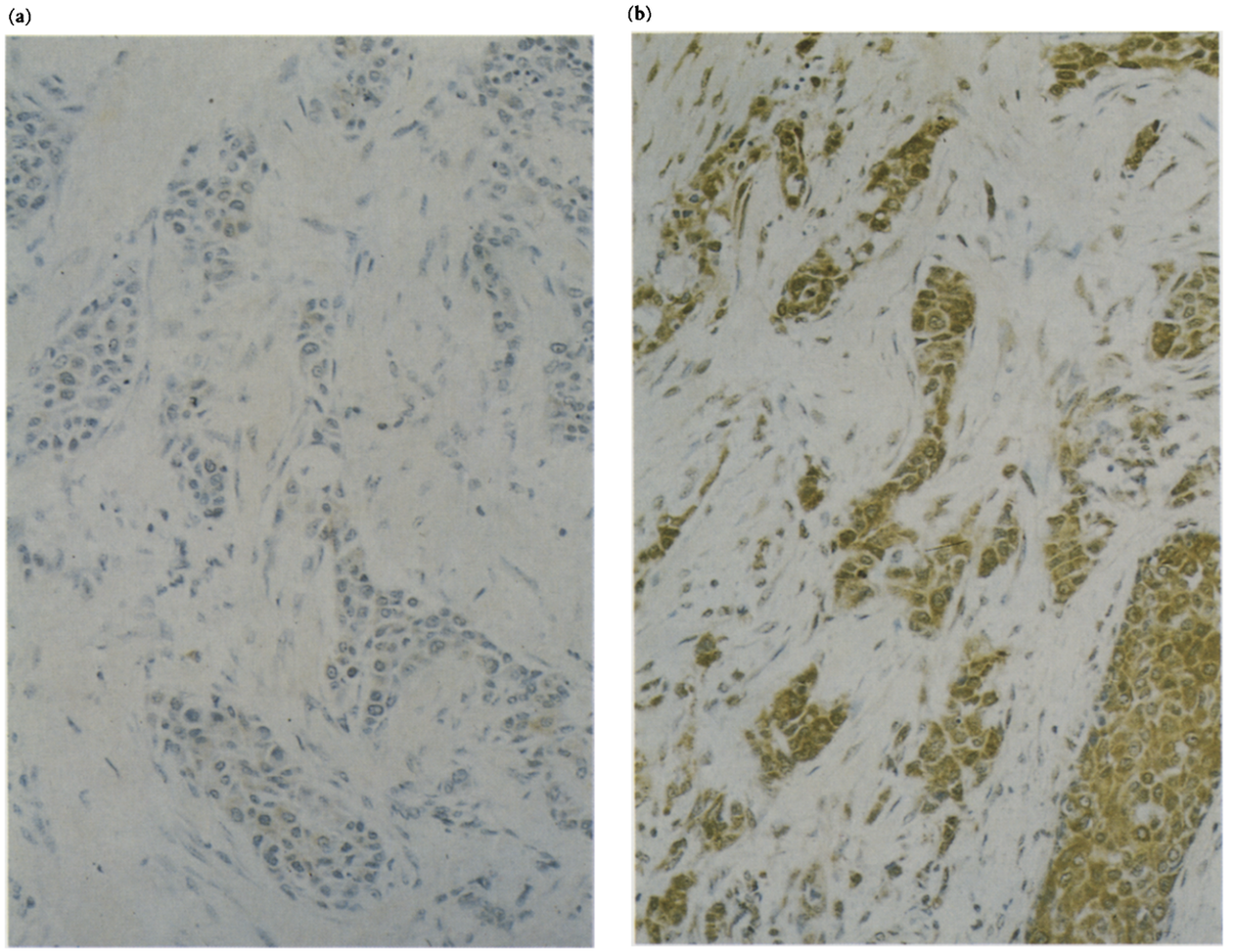


Figure 1. Immunohistochemical analysis of the expression of hydroxysteroid ST in breast tumours. Sections of breast tumour were prepared as described in Materials and Methods, and immunostained with IgG prepared from pre-immune rabbit serum (a), or from anti-(rat liver HST) antiserum (b–d) (overleaf). Figures a and b were photographed at a magnification of ×160, and c and d (from a different specimen) at a magnification of ×250. a and b are fields from a poorly differentiated ductal carcinoma, grade 2. (a) stained with pre-immune IgG is immunonegative, while b shows typical cytoplasmic immunoreactivity in the carcinomatous epithelium. (c) and (d) are views of a different tumour, grade 3 poorly differentiated ductal carcinoma, also with strong immunostaining over carcinomatous cells. A normal breast lobule is included in c, over which there is very light focal immunostaining, chiefly on myoepithelium. A normal breast ductule on d has no immunostaining over the lining epithelium, but light focal staining on the myoepithelium.

ing neoplastic epithelium invading a ductal space could be seen adjacent to normal ductal epithelium. The epithelium lining cystic spaces of fibrocystic disease was also negative. Macrophages, when present, generally showed a light positive staining, but lymphocytes and plasma cells were entirely negative.

These results indicate that HST is expressed at very low levels in the normal terminal duct lobular unit of the human, and that its expression is greatly enhanced in tumour cells arising from this epithelium.

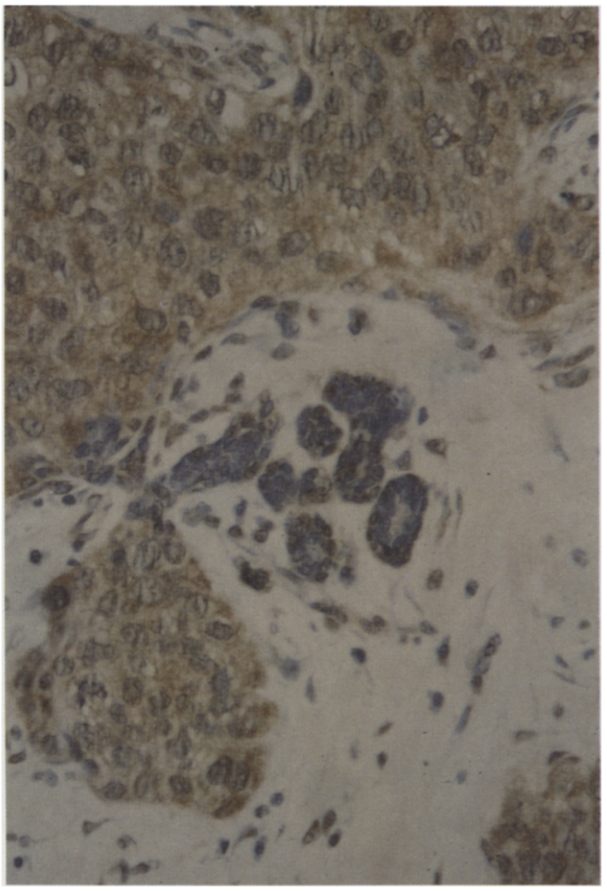
As a positive control experiment to demonstrate the expression of HST in another adult human tissue, sections of normal liver obtained from biopsy were stained with either pre-immune IgG or anti-(rat liver HST) IgG (Figure 2). There was typical cytoplasmic immunostaining in hepatocytes, but little or no staining in other cell types.

DISCUSSION

Androgens, in particular DHEA, are important metabolic precursors of oestrogens, and, as such, are likely to play an important role in hormone-sensitive tumours [12]. HST is the major ST isoform responsible for the sulphation of androgens, including DHEA, androsterone and testosterone [13]. Sulphation of steroid hormones renders them receptor-inactive, but it is also clear that certain sulphated steroids are substrates for the enzymes of oestrogen biosynthesis (reviewed in [2]). Breast tumour cells are known to have the capacity to synthesise oestrogens, and therefore it is important to understand the role of sulfation of oestrogen precursors in the oestrogen biosynthetic process in breast tumours. In addition, other effects of androgens in breast cancer have been observed; for example, in the MCF-7 breast cancer cell line, androgens (testosterone and dihydrotestosterone) were shown to have anti-oestrogenic effects [13], and long-term feeding of DHEA to female C3H (A^{vy}/a) mice inhibited the spontaneous formation of mammary cancer [14]. We have, therefore, examined this expression of HST in the normal and cancerous human breast using immunohistochemistry.

In all the samples studied, the tumour cells expressed HST at

(c)



(d)

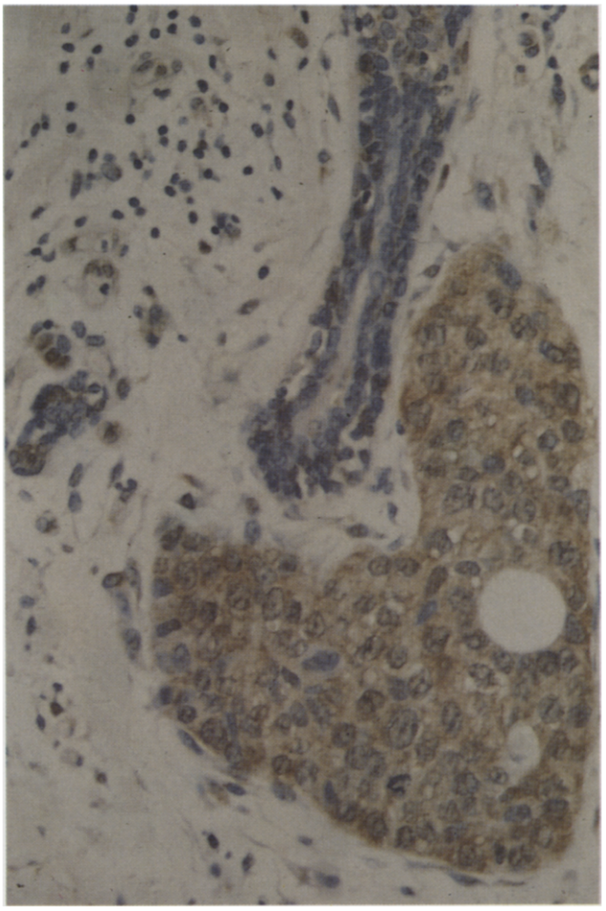


Figure 1. continued.

a significant level, and there was also some weak immunostaining of terminal duct lobular units. However, the epithelial cells lining more distal breast ducts did not react with the antibody which specifically recognises the HST isoenzyme. This observation, therefore, supports the widely held view that, in the majority of breast carcinomas, the neoplastic cells arise from the terminal duct lobular unit [15] and not from ducts as was previously believed. The significant immunostaining over the neoplastic cells indicates that expression of the HST gene is being enhanced at some stage of the carcinogenesis. This is the first demonstration of the expression of this enzyme in breast cancer tissue, as opposed to cell lines. The fact that there did not appear to be any significant difference between the expression of HST in tumours which were oestrogen/progesterone receptor positive or negative is in agreement with investigators who have determined the enzyme activity to be present in human mammary tumour cell lines which are hormone-independent (e.g. MDA-MB-468 [16]) and hormone-dependent (e.g. MCF-7 [17]).

The presence of HST in tumour cells may represent a possible target for novel therapeutic approaches to the treatment of breast cancer. For example, androgens have been used in combination with anti-oestrogens in the treatment of breast cancer, and the results seem to indicate that this combined treatment is more effective than an anti-oestrogen alone [18]. If these findings are borne out, the co-administration of an inhibitor of HST may increase the effectiveness of such therapy, since this would lead to reduced inactivation of the androgen in the tumour cells. We have previously determined that a number of drugs are potent inhibitors of HST activity in human liver cytosol *in vitro* [19].

The implications of the expression of HST in mammary tumour cells for the biosynthesis of oestrogens from their androgenic precursors are not entirely clear. There is some indication that certain steroid sulphates may have a higher affinity for the enzymes of oestrogen biosynthesis than the free steroids—for example, the direct conversion of cholesterol sulphate to pregnenolone sulphate has been shown to occur, and to proceed with lower K_m and V_{max} values than for free cholesterol [20] (cholesterol is also a substrate for the HST isoenzyme [21]). Were the proposed sulphate pathway for steroid biosynthesis [2] to be important in breast tumour cells, blocking of the formation of androgen sulphates may offer therapeutic advantage in combination with, for instance, an anti-oestrogen and an aromatase inhibitor.

We recognise that a study comprising 6 patients must be considered preliminary, and that a wider exploration of normal and diseased breast tissue is now indicated. In particular, there are many different types of breast carcinoma, and it is important to know whether HST is expressed by the whole range or whether negative subsets occur. Examination of benign hyperplasia and pre-invasive carcinoma and the relationships to oncogene expression will also be of interest.

In conclusion, the results presented here indicate that HST expression is enhanced in breast carcinoma cells. The factors

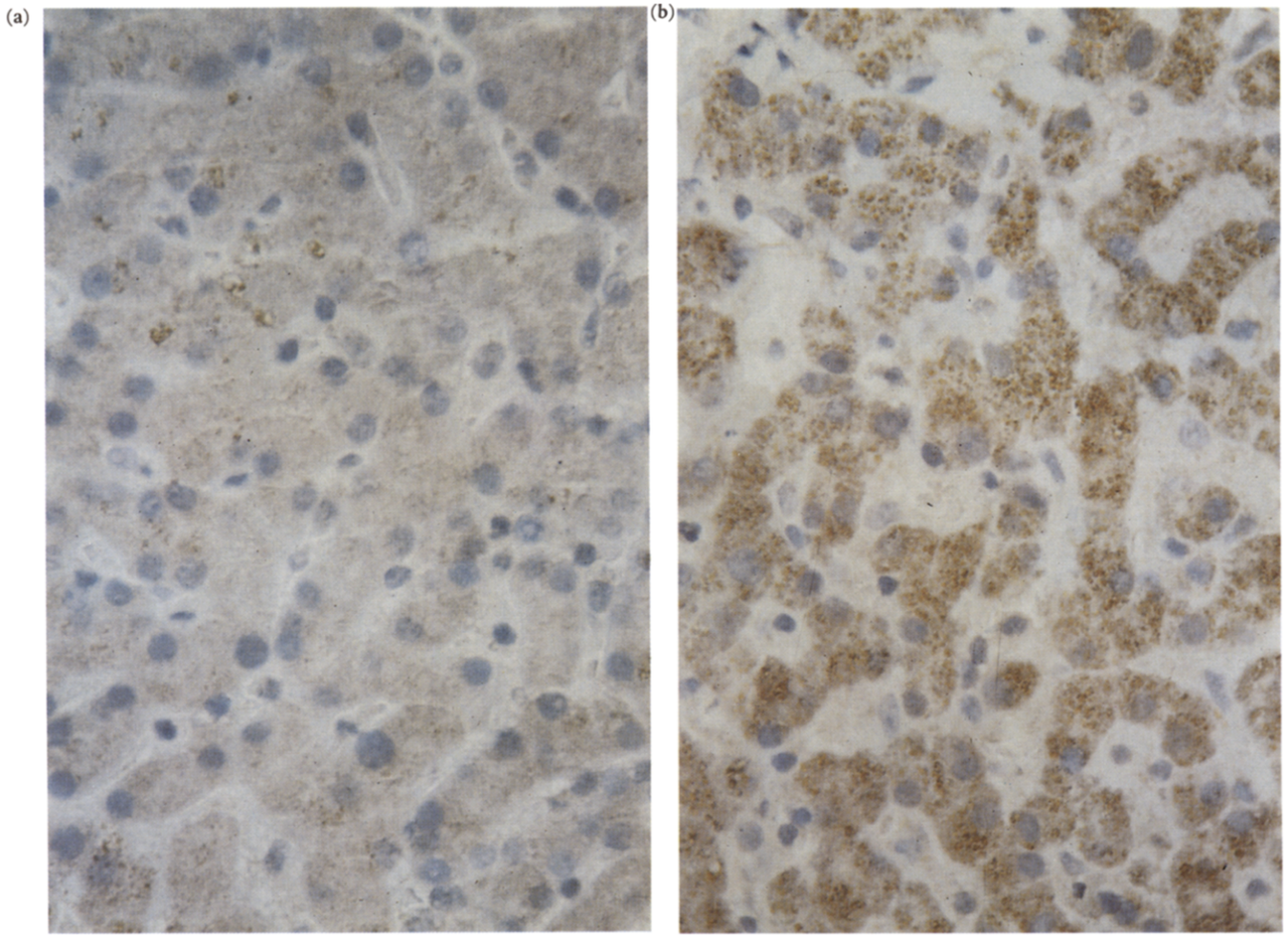


Figure 2. Positive control immunohistochemistry of adult human liver biopsy. Sections were exposed to either preimmune IgG (a) or anti-(rat liver HST) IgG (b). In b, there is typical cytoplasmic immunostaining in hepatocytes, but no significant staining in other liver cell types. Both were taken at a magnification of ×400.

regulating this elevated expression remain to be elucidated, but the observation provides the basis from which future work on the function of this enzyme in breast tumour cells, and the therapeutic potential of interfering with its activity, may proceed.

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Estimating Completeness of Cancer Registration in Saarland/Germany With Capture–Recapture Methods

H. Brenner, C. Stegmaier and H. Ziegler

Completeness of population-based cancer registration has been most commonly quantified by indirect measures, such as the death certificate only index or the mortality/incidence ratio. A major disadvantage of these measures is their strong dependence on the case fatality rate. Capture–recapture methodology offers an approach to estimate completeness directly which does not share this limitation. In this paper, a three-sources modelling approach is employed to derive estimates of completeness for the population-based cancer registry of Saarland. Overall, completeness is found to be high: estimates for all types of cancer range from 95.5 to 96.9% for calendar years 1970, 1975, 1980 and 1985. There is some variation with age (consistently high levels above age 30 years, a minimum of 87.7% in age group 15–29 years) and between cancer sites. Among the most common cancer sites, estimates of completeness are highest for gastrointestinal cancers (97.2%) and breast cancer (97.1%), while lower estimates of completeness are derived for cancers of the female genital organs (92.5%), the urinary tract (91.8%) and the prostate (91.0%). Although capture–recapture estimates are sensitive to the underlying assumptions about dependence between sources, careful application is encouraged to supplement traditional methods for evaluating completeness of cancer registration.

Key words: cancer registries, capture–recapture methods, completeness, epidemiological methods
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INTRODUCTION

THE COMPLETENESS of case ascertainment by population-based cancer registries is most commonly measured by the death certificate only index (DCO index) or by the mortality/incidence ratio (M/I ratio) [1]. For example, these measures are routinely employed as indicators of completeness in *Cancer Incidence in Five Continents*, a widely used series of volumes providing compilations of cancer incidence data from population-based cancer registries all over the world [2–5]. The DCO index quantifies the proportion of cases which are identified exclusively on the basis of the death certificate. It is an indirect measure of completeness which strongly depends, among other things, on the case fatality rate of cancers. The same limitation obviously applies to the M/I ratio. Nevertheless, these measures can be useful for comparing completeness of registration for cancers with similar case fatality rates.

However, it may often be preferable to obtain direct estimates of completeness of cancer registration which do not depend on case fatality rates. Such estimates are straightforward in situations in which there is an independent source of case

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