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Malignancies Associated with Gynecological cancer

- Epidemiological and etiological aspects

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Epidemiology is the simplest and
most direct method for studying
the causes of disease in humans.

Charles H. Hennekens and Julie E. Buring,
in "Epidemiology in medicine" (Little, Brown: Boston 1987)

ABSTRACT

The aims of this thesis were to analyze the incidence and risk of second primary malignancies (SPM) in a Swedish cohort of gynecological cancer patients, to explore suggested risk factors associated with second primary malignancies in ovarian cancer patients, and to evaluate the risk of ovarian cancer in a cohort of breast cancer patients in relation to family history of breast or ovarian cancer.

Incidence of second primary malignancies in 15,200 gynecological cancer patients were investigated in a register-based study and compared to age- and calendar-specific incidence for women in the general population. Increased risk of leukemia was found after ovarian and endometrial cancer. Additional sites of excess risk in ovarian cancer patients were breast, endometrial, colon, rectum, and bladder, while women with endometrial cancer were documented having an increased risk of cancer of the colon, ovaries, vulva, and bladder. Cervical cancer patients were found with increased risk of cancer of colon, rectum, lung, vulva, kidney, and bladder (Paper I). Validity of register data was investigated by comparisons with hospital records for 347 women registered with ovarian cancer and one or more second primary malignancy. Errors in cancer registrations were revealed concerning the first as well as the second primary cancer, although previously risk estimates remained increased when corrected for registration errors (II). In a multi-center case-control study, platinum-based chemotherapy was shown to be highly associated with an increased risk of leukemia (III). The risk of second primary breast cancer in women with ovarian cancer was associated with heredity, nulliparity, and late menopause. Furthermore, 43 % of the breast cancer cases were diagnosed without symptoms of the disease in line of routine follow-up, indicating clinical surveillance to be important when reflecting the incidence of SPM (IV).

In order to further investigate family history as a risk factor, the risk of ovarian cancer in breast cancer patients was analyzed using data from the Swedish Generation Register. Breast cancer patients with a family history of breast or notably ovarian cancer were found to be at high risk of subsequent ovarian cancer (V).

The results confirm that women with gynecological cancer have an increased risk of second primary malignancies at certain sites, knowledge that should be considered in clinical follow-up. In ovarian cancer patients, a part of the excess risk of subsequent malignancies could be referred to register errors and intense clinical surveillance. Regarding the appearance of ovarian cancer as a second primary malignancy in breast cancer patients, the risk was found to be high in young women with a family history of breast or ovarian cancer, implementing the need of intense clinical surveillance in high-risk groups, even considering prophylactic oophorectomy in selected cases.

Key words: neoplasms, second primary, ovarian cancer, breast cancer.

LIST OF PUBLICATIONS

1.

This thesis is based on the papers listed below which will be referred to by their Roman numerals:

I. Bergfeldt K, Einhorn S, Rosendahl I, Hall P. Increased risk of second primary malignancies in patients with gynecological cancer. A Swedish record-linkage study. *Acta Oncol* 1995, **34**, 771-777.

II. Bergfeldt K, Silfversward C, Einhorn S, Hall P. Overestimated risk of second primary malignancies in ovarian cancer patients. *Eur J Cancer* 2000, **36**, 100-105.

III. Travis LB, Holowaty EJ, Bergfeldt K, Lynch CF, Kohler BA, Wiklund T, Curtis R, Hall P, Andersson M, Pukkala E, Sturgeon J, Stovall M. Risk of leukemia after platinum-based chemotherapy for ovarian cancer. *N Engl J Med* 1999, **340**, 351-357.

IV. Bergfeldt K, Nilsson B, Einhorn S, Hall P. Breast cancer risk in ovarian cancer patients - a case control study. *Eur J Cancer* 2001, **37**, 2228-2233.

V. Bergfeldt K, Rydh B, Granath F, Grönberg H, Talib L, Adami H-O, Hall P. Increased risk of ovarian cancer in breast cancer patients with a family history of breast or ovarian cancer (submitted for publication).

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CONTENTS

LIST OF ABBREVIATIONS

AR	- absolute risk
BC	- breast cancer
BRCA-1	- breast cancer gene No. 1
BRCA-2	- breast cancer gene No. 2
CI	- confidence interval
HNPCC	- hereditary non-polyposis colon cancer
HRT	- hormonal replacement therapy
OC	- ovarian cancer
OR	- odds ratio
RR	- relative risk
SCR	- Swedish Cancer Registry
SIR	- standardized incidence ratio
SPM	- second primary malignancies

1 INTRODUCTION

The incidence of second primary malignancies (SPM) in cancer patients is increasing, in Sweden as well as worldwide (1, 2). This increase is likely to be explained by improved survival, due to improvements in treatment and earlier detection of various malignant diseases. In 1998, Swedish cancer statistics reveal that malignancies found in individuals with previous cancer diagnoses were more numerous than ever before (Figure 1). More than 6,000 of all incident cancer diagnoses were found in individuals previously registered with malignant diseases.

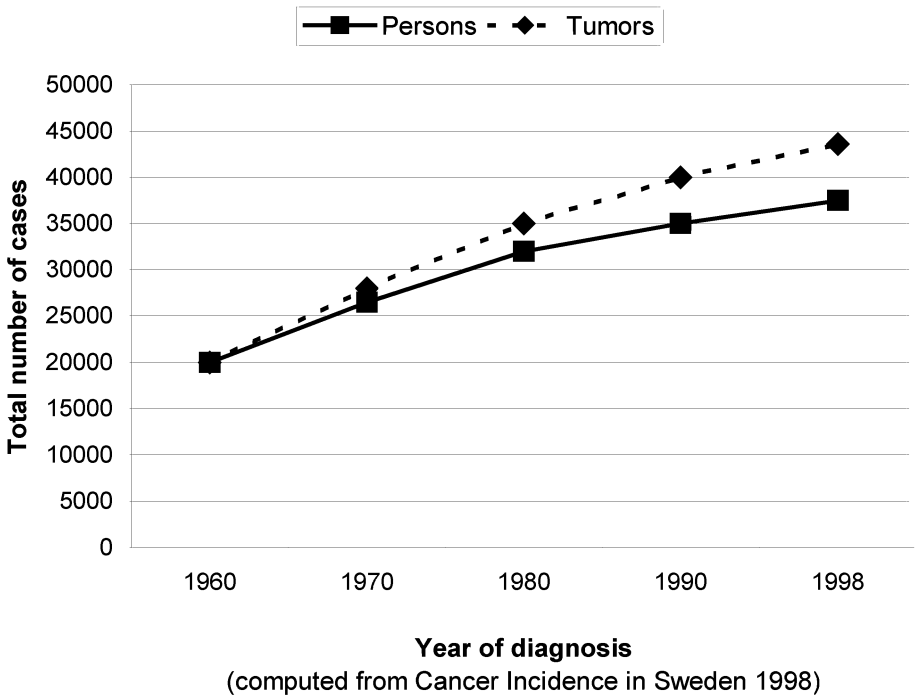

Figure 1. Incidence of new cancer cases per year in Sweden from 1960 to 1998 and number of individuals diagnosed with cancer during the same time period.

Gynecological cancer constitutes a group of malignant diseases with a yearly incidence of nearly 3,000 cases in Sweden. Women with gynecological cancers, experience an increased risk of developing second primary malignancies, according to several large population-based studies (3-5).

In the clinical setting, when a patient is presented with more than one severe disease, most clinicians – and probably most patients – are faced with a number of questions: Are there links between these diseases? Are they related? How are they related?

Epidemiological studies have provided evidence of numerous risk factors associated with occurrence of second primary malignancies. The aim of this thesis was primarily to investigate, and to some extent quantify, these risk factors and relations between the first primary and subsequent malignancies in gynecological cancer patients.

1.1 HISTORY

When the German surgeon Billroth presented a case of multiple malignancies in 1891 he was the first in a line of scientists focusing on relations between different malignant diseases (6). However, throughout the first half of the 20th century, knowledge in the field of multiple malignancies developed slowly. Additional case reports and case series were published but they were all hampered by lack of evidence on significant biologic associations. Since so little was known about the cancer incidence in the general population, there was no feasible way to show that a second primary tumor occurred more frequently than might be expected by chance. Nevertheless, the case reports were useful in defining and documenting the phenomena (7).

Case-control studies and larger hospital based series followed and increased the knowledge by identifying neoplasms frequently occurring together in the same patient, indicating biological associations. Still, since the problems with defining adequate control groups remained, it was not possible to rule out that these observations were due to chance.

When population-based cancer registries were introduced in the middle of the 20th century, it became possible to systematically investigate the occurrence of multiple

malignancies. The first reports came in the early 1960s and numerous epidemiological studies have followed, making it possible to distinguish relationships between different primary malignancies and certain sites of SPM (7). It became evident that gynecological malignancies were frequently associated with an increased incidence of SPM at certain sites (3, 4, 8-13). Simultaneously, the discussions on how to explain this co-existence of more than one malignancy in the same individual became more intense. By time, the expanding knowledge in the field of carcinogenesis could be adapted to the issue of second primary malignancies. As was the opposite, knowledge on carcinogenesis was expanded due to studies on associations between first and second primary malignancies.

1.2. GYNECOLOGICAL CANCER

Gynecologists and gynecological oncologists deal with a limited number of cancer diseases, however the properties of these cancers are quite different, as are the incidence of different gynecological cancer. In all, 2,936 cases of gynecological cancer were registered in Sweden 1998 (14 % of female cancer). The dominating sites are cervix uteri (n = 432; 15 %), corpus uteri (n = 1,300; 44 %), and ovaries (n = 850; 29 %). Cancer of the vulvae, vagina, and the fallopian tubes are rare and constitute together 12 % of all gynecological cancers. Age distributions of incident cases of the major gynecological cancers are given in figure 2 (1). In comparison, incidence in Sweden is similar to other countries in Western Europe and the US, where incidence of ovarian and endometrial cancer are among the highest in the world (10-15 cases per 100,000). In developing countries incidence of ovarian and endometrial cancer is lower, probably reflecting different age distribution of the population. The pattern is opposite when studying the incidence of cervical cancer, high in developing countries and low in the western world, including Sweden (14).

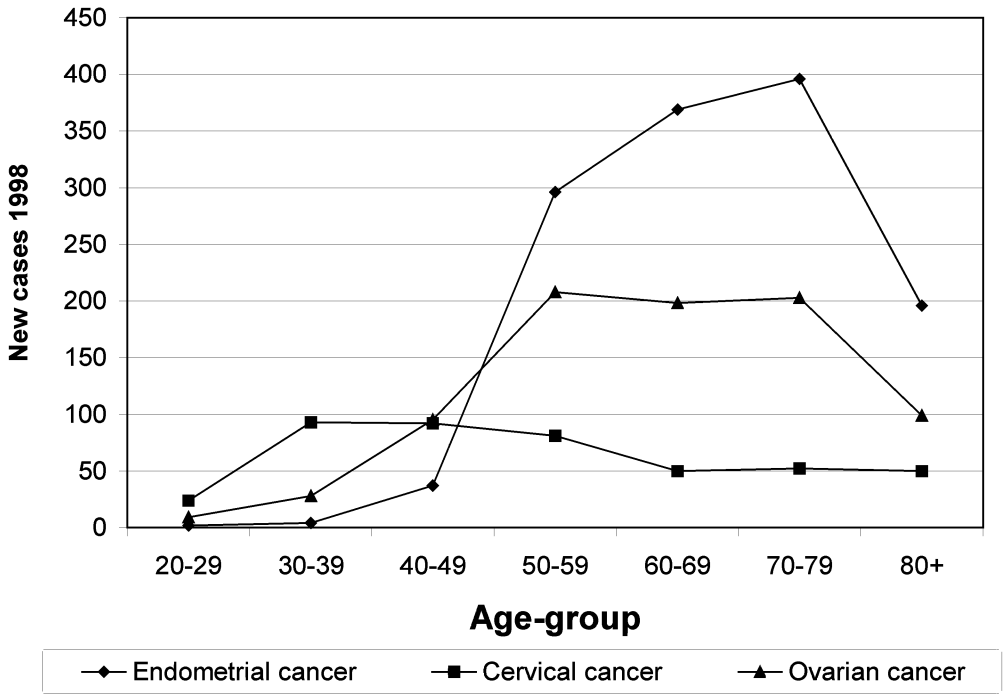

Figure 2.
Incident cases of gynecological cancer in Sweden 1998 by age at diagnosis (1).

1.2.1. Cervical cancer

In a global perspective cervical cancer seem to have two properties. In the western world the incidence and mortality has decreased during the second half of the 20th century (14), while in developing countries cervical cancer continue to be one of the most common cancers in women. Mortality rates are high, mainly attributable to the fact that most cases are diagnosed in advanced stages (15). The improved survival in western countries is widely recognized to be a consequence of improved treatment and the screening programs introduced during the 60s and 70s (16-18).

Two histological subgroups of cervical cancer can be distinguished. Squamous cell carcinoma is by far the most common, constituting more than three-quarters of all cases, while adenocarcinoma accounts for additional cases. Contrary to squamous cell

carcinoma, adenocarcinomas show an increasing incidence over time (19, 20). The reason for this is obscure and can only partially be explained by the fact that screening techniques used so far are unlikely to discover precancerous conditions related to adenocarcinomas. Additionally, use of oral contraceptives has been suggested as a risk factor for adenocarcinomas of the cervix and might be part of the explanation of increasing incidence (19, 21).

Early in the history of epidemiology, sexual behavior was established as a risk factor for cervical cancer (22). Cervical cancer was rare among nuns and more frequently appearing among prostitutes, indicating a sexually transmitted carcinogenic determinant. Several hypothesis were tested but it was not until the development in molecular biology provided the tools for genetic and serological testing that the link to human papilloma virus (HPV) became evident (23). Today it is widely accepted that HPV-infection is a causal factor in cervical cancer development. Additional risk factors are described and most likely act as co-factors for malignant transformation together with HPV. Smoking, multiparity, oral contraceptive use, and impairment of the cellular immune system are factors suggested as independent risk factors of cervical cancer but might also act as co-factors to HPV (21, 24).

In Sweden, as well as in and most other developed countries, surgery and radiotherapy are the most widely used treatment modalities in cervical cancer. In most cases, the diagnostic pathway from a pathological cytological specimen goes via cervical curettage and minor excision of suspected areas of the portio, to conisation where the distal part of cervix is removed. If there is evidence of remaining malignant tissue, extended surgery is performed. In most cases, surgery includes hysterectomy and sampling of pelvic lymph nodes, followed by radiotherapy when there are signs of advanced disease. In cases initially considered advanced, treatment starts with intracervical radiotherapy (brachy-therapy), and when feasible, surgery is performed. Occasionally, in advanced stages or relapses, chemotherapy is added (25).

1.2.2. Cancer of the corpus uteri

In this thesis, endometrial cancer is used synonymous with cancer of the corpus uteri and covers the major pathological features of cancer at this site, that is, cancers arising in the endometrial epithelium. Additionally, mesenchymal malignancies, sarcomas, account for less than five percent of all malignancies of the uterus. Endometrial cancer is a disease of

the elderly women, less than three percent of all cases are found in women younger than 50 years, while 46 % occur in women 70 years or older (Figure 2). Corresponding incidence rates are less than two cases per 100,000 at 40 years of age and 100 per 100,000 at 70 years. In cervical cancer for comparison, 48 % of all cases occur before the age of 50 years (1). Globally, incidence of endometrial cancer is high in North America and Europe, while low rates are found in developing countries (14).

The etiology of endometrial cancer is closely linked to effects of circulating estrogens stimulating epithelial cells of the endometrium to increase mitotic rate, which lead to more frequent DNA-damage and less time for repair (26). The pathological development goes from hyperplasia, via atypical changes to neoplasia. By far, the most common histological subgroup of endometrial cancer is endometroid adenocarcinoma, constituting 90 % of all tumors. There is a handful of additional adenocarcinomas, which all is related to a poor outcome, while in general, endometrial cancer has a comparatively good prognosis. Five-year survival is 80 % and this relatively favorable prognosis is most likely explained by early detection due to symptoms like bleeding, which is a frequent early symptom of the disease, and brings the women to clinical counseling.

Risk factors for endometrial cancer are closely linked to determinants related to endogenous or exogenous estrogen exposure. Reproductive factors, such as nulliparity and late age at menopause, together with hormonal replacement therapy (HRT) with unopposed estrogen (that is, without addition of continuos or sequential progestins) are well-established risk factors (26-30). Estrogen exposure and subsequent risk of endometrial cancer could also be understood from associations established with estrogen-producing tumors of the ovary (that is, granulosa cell tumours) and treatment with Tamoxifen, frequently used in breast cancer patients with a proven estrogen-like effect on the endometrium (31, 32).

Treatment of endometrial cancer is primarily surgical, added with radiotherapy when there are signs of advanced disease. Hormonal treatment with progestins opposing the effect of estrogens in the endometrium has also been proven efficient, and included in the therapeutic considerations in advanced cases, as well as in women unsuitable for surgery due impairment of other diseases or high age. Until recently, the majority of Swedish endometrial cancer patients were given intrauterine radiotherapy before surgery, but today

surgery is first-line therapy most often preceded by curettage or other techniques for ascertaining histopathological specimens (25).

1.2.3. Ovarian cancer

There are numerous histopathological features of ovarian cancer. Epithelial ovarian cancers constitute the majority (90 %), and are classified as being either borderline (of low malignancy potential) or malignant. Depending on pathological features, epithelial tumors are further classified as serous papillary, mucinous, endometrial, clear-cell, or poorly differentiated, where the variability is explained by the embryological origin of the ovarian epithelium. In addition to the tumors arising in the surface epithelium of the ovaries, there are several groups of non-epithelial tumors together constituting close to ten percent of all ovarian malignancies. Those tumors are markedly different from epithelial tumors in terms of carcinogenesis, risk factors, treatment, as well as prognosis, and will not be addressed further in this thesis.

Prognosis in epithelial ovarian cancer is strongly related to stage at diagnosis, where localized disease has a fairly good prognosis with a five-year survival close to 80 %. Unfortunately, and contrary to endometrial carcinoma, the disease seldom gives rise to early symptoms. Therefore, a minority of all cases (25 %) are presented at early stages and the overall survival in Swedish ovarian cancer patients is 37 % (33). Any strategy for early detection of ovarian cancer would therefore be beneficial in terms of increased survival, but so far there are no screening modalities available providing this opportunity (34-36).

Contrary to endometrial and cervical cancer, the etiology of ovarian cancer is obscure. The incessant ovulation-theory (37) suggest that cancer risk is associated with numerous ovulation causing multiple damage to ovarian epithelium with an increasing probability of DNA-damage. The gonadotropin-theory (38) explains ovarian cancer risk as an effect of continuos gonadotropin-stimulation to the ovarian epithelium. These models are more or less well fitted to risk factors suggested to be associated with ovarian cancer, such as age, early menarche, late menopause, and nulliparity (39-43). Another well established risk factor is family history of breast and ovarian cancer (44). Among the hereditary syndromes associated with an increased risk of ovarian cancer, the highest incidence is found in the hereditary breast and ovarian cancer syndrome linked to inherited mutations in the BRCA-1 and BRCA-2 genes. Ovarian cancer incidence is also increased among

patients with the hereditary non-polyposis colon cancer, also known as the Lynch II-syndrome where, besides colon cancer, endometrial cancer is frequently appearing (44). Additionally, ionizing radiation seems to be associated with incidence of the disease. A moderate excess risk was found in atomic bomb survivors. Hence, ionizing radiation is suggested as a possible causal agent, an association that was further proven in studies of late effects of radiotherapy in women treated for cervical cancer (45-47). Additionally, decreased numbers of ovarian cancer is associated with use of oral contraceptives, tubal ligation, and hysterectomy (48-50).

Ovarian cancer treatment is primarily surgical, and in most cases added with chemotherapy using combinations of platinum-based drugs, in recent years added with paclitaxel, which both have contributed to a small improvement in ovarian cancer survival (33).

1.2.4. Time trends

Following incidence of gynecological cancer over time reveals dramatically decreasing incidence of cervical cancer, a smaller decrease of ovarian cancer, and increasing incidence of endometrial carcinoma. Nation-wide screening programs with regular Pap-smear provided all Swedish women from 25 years of age are widely accepted to account for the decline in cervical cancer incidence, where age specific incidence has been reduced by more than 50 % from 1965 to 1998. Simultaneously, incidence of precancerous lesions (cancer in situ, stage 0, or CIN III) has increased and in 1998, 3,082 cases were registered (1, 18, 51). In ovarian cancer, decreasing incidence might be expected as an effect of oral contraceptives introduced in the 1960s and proven to reduce ovarian cancer incidence. (49, 52). Accordingly, the trend of annually increasing incidence found in the years 1960-75 was halted and from 1979 the annual age specific incidence per 100,000 has dropped from 24.2 to 17.2. However, further decrease might be opposed by increasing numbers of elderly women in the society.

Contrary to cervical and ovarian cancer, cancer of the corpus uteri is becoming more numerous, with increasing rates documented for the last 20 years (age standardized incidence per 100,000 increased from 21.3 in 1979 to 25.6 in 1998) (1). Primarily suggested explanations for this increase are both the fact that the population is growing older and the wide-spread use of unopposed estrogen provided peri- and postmenopausal women in the 1960s and 70s.

1.3. SECOND PRIMARY MALIGNANCIES

For several reasons, the issue of second primary malignancies has been given increasing attention within the last decades. One major reason is the possibility to address questions concerning cancer etiology, provided by cases of multiple malignancies. Furthermore, incidence of second primary malignancies is increasing (1, 2), most likely due to increased survival in many malignant diseases, making knowledge of determinants associated with risk of second primary malignancies increasingly important, and finally, development in epidemiological methodology has provided tools to address the issue.

By definition a second primary malignancy should be possible to distinguish from the first primary, by the occurrence at a different site or with different morphology, and maybe most important, the possibility that the new tumor is a recurrence or metastasis of the first primary should be ruled out. These fundamental features of second primary malignancies are the corner stones in the most widely accepted definitions for classification of second primary malignancies presented by the International Agency for Research on Cancer (IARC) (53), which with some amendments is also used in Sweden. The amendments are stated in governmental legislation concerning registration of benign conditions with some malignant potential, for instance, ovarian thecoma.

Increasing incidence of second primary malignancies are suggested to be a result of improved survival achieved by better treatment and earlier detection (2). Accordingly, the major explanatory factor behind this increase would be additional time at risk, which is likely to increase the possibility to develop any age-related diseases, for instance an additional neoplasm. Another determinant of increasing incidence would be treatment of the first cancer. Both chemo- and radiotherapy are well-established carcinogens and frequently used in oncologic treatment. Therefore, with increasing survival rates, adverse effects of cancer treatment are likely to appear more frequently. An additional effect of improved survival might also be that second primary malignancies sharing etiological properties with the first primary would be given more time to develop.

1.4. ETIOLOGICAL CONSIDERATIONS

Naturally, the fundaments of etiological considerations in the issue of second primary cancers are associated with corresponding considerations on the primary malignancies involved. In fact, studies of associations between first and second primary malignancies

have made substantial contributions to the knowledge of carcinogenesis for several sites of cancer; notably hereditary cancer syndromes (54) and treatment induced malignancies.

1.4.1. Heredity

Familial predisposition to cancer is generally viewed to be associated with the transfer of a defective gene with the potential to increase cancer susceptibility (55). The inactivated - or damaged - gene is present in every cell of the body, which makes the concept of multiple malignancies occurring in members of cancer prone families understandable. Although cancer is a result of multi-step development where a number of genetic changes have to be present, an individual with an inherited mutated gene is at increased risk of cancer, since that gene represents one crucial step in carcinogenesis. One damaged genetic allele is just one step away from the complete loss of a gene. However, the risk for an affected individual to develop the disease associated with the inactivated gene – known as the penetrance of a gene - varies widely and may be modified by other genes, as well as other exogenous or endogenous factors.

Besides tumor suppressor genes, which are the most common genes related to hereditary cancer syndromes, there are genes associated with DNA-repair and proto-oncogenes among the more than 20 different syndromes defined. Collectively, the hereditary cancer syndromes have been estimated to affect approximately five percent of all cancer patients (55, 56).

Hereditary non-polyposis colon cancer (HNPCC), associated with damage to DNA-mismatch repair genes, and hereditary breast and ovarian cancer, associated with BRCA-1 and BRCA-2 (both characterized as tumor suppressor genes) are syndromes associated with susceptibility to gynecological cancers (44, 57-59). In the former, colon cancer is the most prominent feature together with endometrial and ovarian cancer, while in the latter breast cancer dominates over ovarian cancer. Additionally, other types of cancer are found in members of affected families. However, it should be noticed that mutations in BRCA-1 or BRCA-2 are present in small numbers of all breast cancer cases (60-62) and they seem to account for only a limited fraction of all breast cancers with a genetic component (63)).

The incidence of second primary malignancies is prominently increased in members of cancer prone families. Breast cancer patients documented with mutations in BRCA-1 or

BRCA-2, are found with a ten-fold relative risk of subsequent ovarian cancer. Furthermore, breast cancer patients with mutations in BRCA-1 experience a close to 50 % absolute risk of developing subsequent ovarian cancer before the age of 70 years, while in patients with mutations in BRCA-2 absolute risk estimates seem to be smaller (64-66).

According to a recently published study using the Swedish Generation Register, colon cancer patients belonging to HNPCC-families experience more than ten-fold rates of second primary endometrial and ovarian cancer in comparison with population-based incidence (67).

1.4.2. Common etiology

Smoking is the number one risk factor for cancer in the Western world and associated with more than every fourth cancer (68). Among the sites of cancer found in excess among smokers are lung, bladder, and cervical cancer. (69). Besides smoking, numerous risk factors associated with more than one malignancy have been described these risk factors are likely to account for some of the excess risk with regard to second primary malignancies.

In females, breast, endometrial, and ovarian cancer share the etiological properties of hormonal carcinogenesis, that is, the cells are under influence of endogenous or exogenous sex hormones, mainly estrogens and gestagen (26, 52, 70-72). Sex hormones are known to drive cell proliferation, and thus the possibility of accumulation of genetic errors and subsequent malignant transformation is increased. Several large population-based studies have provided evidence that women with breast, ovarian or endometrial cancer are at increased risk to develop each one of the other cancers, suggesting a common hormonal etiology (3, 4, 8, 10, 73-76). Age at menopause is one example of this association, where increasing age is proven proportional to the risk of all three, and suggested as a proxy for estrogen-exposure in a woman during a substantial period of her life. Another example would be cancer risk associated with hormonal replacement therapy, provided to numerous peri- and postmenopausal women and strongly related to increased risk of breast cancer (77-79). Initially, in the 1960s, this treatment was given with estrogen as single drug, resulting in a highly significant increase in endometrial cancer incidence (28, 29). By time, supplement of progestins was introduced and since then, the excess risk has decreased. However, progestins has not been found with the same protective effect on breast cancer risk, where even an additive risk associated with

progestins is suggested (78). Ovarian cancer risk in association with hormonal replacement therapy is not that well described, and the results are conflicting (52).

Hormonal effect and carcinogenesis is further proven by the use of Tamoxifen in postmenopausal breast cancer patients and subsequent increase of endometrial cancer incidence. Tamoxifen acts as an anti-estrogen in mammalian tissue, while in the endometrium of the uterus Tamoxifen has an estrogen-like effect, stimulating the endometrium, and as an adverse effect, increases the risk of cancer (Rutqvist).

Immunological factors

However disputed during the last decades of the 20th century, another property of common etiology would be the impact of an individual's immune system (80). Despite the doubts, immunodefiency, for instance immuno-suppression in kidney-transplanted patients, is documented to increase the risk of non-Hodgkin lymphoma (NHL) and predispose for skin cancer, including melanoma and several other solid tumors (81). Furthermore, immunological properties have been suggested to act as co-factors in the carcinogenic action of infectious agents associated with several malignancies (82). Among those well-described agents are human papillomaviruses (HPV), documented as causal agent in cervical cancer (as well as in other ano-genital cancers) (23). Additionally, Helicobacter pylori, known to be causal agent in gastric ulcers, has been proven to be an important factor for gastric cancer, hepatitis B-virus is linked to liver cancer, as are hepatitis C-virus. Epstein-Barr-virus have been linked to B-cell malignancies and nasopharyngeal cancer (83-85).

In consequence, reciprocal concerns on properties of the immune system as a risk factor of second primary malignancies are suggested by the findings that women with cervical cancer experience an increased risk of cancer at the other sites etiologically linked to infections with human papillomaviruses, namely vagina, vulva, and anal cancer (86).

1.4.3. Treatment

Increasing survival rates in several cancers are partly due to advances in the use of chemo- and radiotherapy. Improved cancer survival is most evident in childhood and early adulthood cancers, for instance testicular cancer, where survival and mortality rates were completely altered with the introduction of modern chemotherapy in addition with

radiotherapy in the 1980s. In the earlier years, survival rates were ten percent, while today the relation is opposite, with survival rates close to 90 % (87). In testicular cancer, as well is in all other malignancies, increased survival rates has increased the awareness of treatment-induced malignancies, since both chemo- and radiotherapy are established as powerful carcinogens (88).

Despite this carcinogenic potential, chemo- and radiotherapy cause cancer only in a minority of exposed patients. Therefore, it might be suspected that the risk is modified by individual factors. For chemotherapy, the risk of developing additional cancers would depend on the therapeutic agent, possible interactions between different drugs used in combination and properties of drug-metabolism within the individual cancer patient. Clearance and repair of damaged DNA-molecules are additional factors that might affect the risk of adverse effect of chemotherapeutic agents. Accordingly, inherited impairment of relevant enzyme systems could therefore create a genetic susceptibility to second malignancy development (89). Reciprocal concerns could be adapted to radiotherapy, since radiation-induced carcinogenesis might interact with other exogenous or endogenous factors (90).

Chemotherapy

Most agents used in chemotherapy for cancer-treatment are carcinogenic in animal models, inducing hematological as well as solid malignancies. However, in humans, leukemia is found to be the primary feature of chemotherapy-related second primary malignancies and found in excess after treatment with most types of established agents (89, 91). Both alkylating agents and epipodophyllotoxins have been causally related to leukemia, and the former has for several decades been widely used in gynecological cancer treatment, whereas the latter was introduced more recently and is not yet frequently used. Contrary to hematopoetic disorders, solid tumors are rarely found in association with chemotherapy, with the exception of bladder cancer, which in several studies have been found in excess in patients treated with cyclophospahmide (an alkylating agent) (92, 93). Additional excess risk of solid tumors has been suggested but any strong association has been difficult to verify (94-96).

Radiotherapy

Beside tobacco, ionizing radiation is probably the most studied carcinogen in humans. Study populations having provided the largest amount of knowledge are survivors of the

Japanese atomic bombs and cohorts of patients exposed to radiotherapy (46, 47, 97-102)). Radiotherapy is both historically and to date widely used in the treatment of cancer at different sites, including gynecological cancer. In women with cervical and endometrial cancer radiotherapy is frequently used together with surgery. For patients not fitted for surgery, radiotherapy is the single treatment of choice (25, 26). Treatment is given both as external beam radiotherapy, using a source outside the body directed towards the pelvic region and lower abdomen, and as brachytherapy, where the source of radiation is placed inside the cervix or uterus. At present, radiotherapy is not widely used in ovarian cancer patients, whilst in the 1960-70s it was more common and then given towards the lower abdomen, eventually including the total abdominal cavity (25, 76, 103).

Ionizing radiation causes damage to the DNA-helix through direct effects on the molecule or indirect via free radicals produced as a consequence of ionizing radiation, making the carcinogenic potential of radiation almost intuitively understood. However, since far from all individuals exposed will develop cancer, exposure to ionizing radiation seems to be a causal, albeit not sufficient, factor for carcinogenesis, and presumably acts through interaction with other risk factors. Hence, mechanisms of radiation-induced malignancies are complex, and beyond the scope of this thesis. In brief, complexity could be understood by the numerous factors and co-factors that might act or interact in malignant transformation. Beside actual dose, several properties of radiation are likely to be important, for instance the type, energy, and rate of radiation. Different tissues of the human body are proven with various sensitivity to ionizing radiation and there are also a wide range of differences in individual susceptibility, that is, individual differences in capacity of managing damage caused by radiation, for instance repair of damaged DNA. Another factor interacting with cancer risk and radiation is age at exposure, where exposure in young age and early adulthood is likely to be more deleterious than in old age.

Most of our knowledge on cancer risks after radiation comes from the survivors of the atomic bombs in Hiroshima and Nagasaki. Noticed excess cases among the survivors compared to unexposed Japanese citizens has given information on what sites might be connected with radiation-associated malignancies. Cancers most easily caused of radiation are thyroid, breast, and lung together with bone marrow-derived malignancies as acute leukemia and chronic myelocytic leukemia (46, 90, 104). Risk in relation to site of cancer found in follow-up studies of atomic bombs survivors are given in Figure 3.

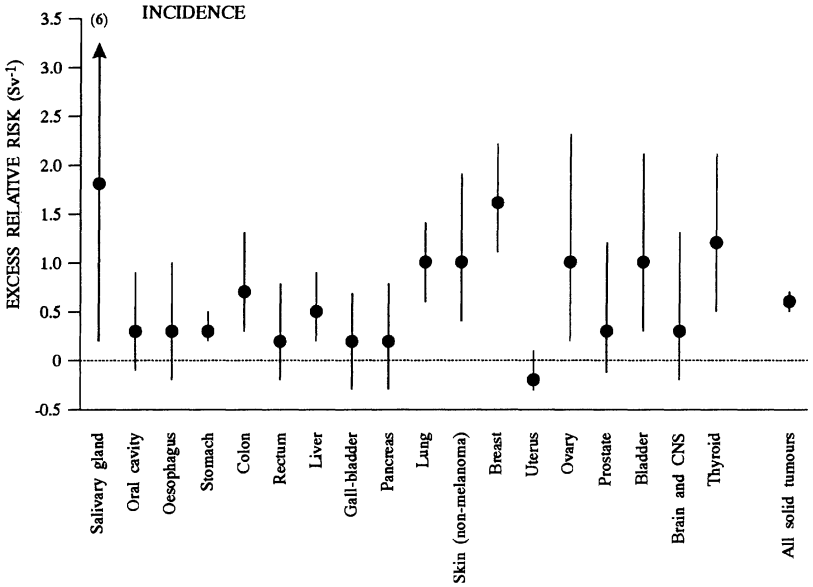

Figure 3. Sites of solid tumors and relative excess risk in relation to absorbed dose of radiation in survivors of the atomic bombs (46).

Additional knowledge has been provided by follow-up studies on patients that have been given radiotherapy for benign and malignant conditions (47, 97-101). Therefore, the pattern of second primary malignancies after gynecological cancer is quite vigorously described. Large cohort studies have been able to establish a small excess risk of leukemia due to radiotherapy, contrary to chemotherapy-related second primary malignancies, where excess risk of leukemia is profoundly affected by treatment, whereas the occurrence of solid tumors are more strongly linked to radiotherapy. Increased incidence of solid tumors typically starts 5-10 years after treatment with a continued excess risk over several decades. Bladder cancer together with soft tissue sarcomas and rectal cancer are highly associated with radiotherapy, while evidence of excess risk at other sites are suggested but less strongly documented. A high risk of subsequent cancer at the other site

has been described for patients with ovarian and endometrial cancer. However, this association has not been convincingly linked to radiotherapy (104).

1.4.4. Register errors

Population-based cancer registries are fundamental in modern cancer epidemiology, and not less important in the studies of second primary malignancies. Therefore, validity of the registries is crucial and the excellent quality of the Swedish Cancer Register has been proven in several investigations (105, 106). Close to 98 % of all solid tumors are found in the register. Cancer registers in other Scandinavian countries and several other western countries are documented with high validity. However, documented validity of cancer registries concerns primary cancer registration. Very little is known about the validity of SPM-registration. Misclassification is likely to occur and there is a probability that clinicians are less eager to verify a second tumor occurring in patients treated for severe cancer diseases. For instance, second primary colon cancer in ovarian cancer patients could mistakenly be diagnosed and treated as relapse of ovarian cancer – then a true relation between the two malignancies would be underestimated. Contrary, when metastasis of primary ovarian cancer in the large intestine is erroneous diagnosed and registered as colon cancer, a spurious association is established.

1.4.6. Clinical surveillance

It is widely recognized that clinical surveillance increases the possibility to diagnose indolent tumors and tumors of low malignancy potential, tumors that otherwise might never have been discovered. From this follows that in cancer patients, who are more likely to meet physicians and undergo clinical examinations than the average citizen, a fraction of malignancies will be found as a consequence of this surveillance and would bias comparisons with other populations. An example, relevant for this thesis, is women with endometrial or ovarian cancer. Manual breast examinations as well as mammographic x-ray are likely to be included in clinical follow-up, resulting in increased incidence rates, which may bias comparisons with incidence of breast cancer in the total female population. However, from a clinical point of view, early detection – as with primary malignancies - is likely to increase survival possibilities, which would be a rational for performing extensive clinical controls in cancer patients. For the clinicians, studies of second primary malignancies also provide knowledge of where to intensify screening efforts. However, the possible impact of clinical surveillance should be kept in

mind when interpreting studies on second primary malignancies, as well as the possible bias that clinical surveillance would introduce when investigating etiological similarities between the first and second malignancy.

1.5. AIMS OF THE THESIS

The aims of this thesis were

to analyze the incidence and risk of second primary malignancies in a Swedish cohort of gynecological cancer patients,

to explore suggested risk factors associated with different sites of SPM in ovarian cancer patients,

to evaluate the risk of ovarian cancer in a cohort of breast cancer patients in relation to family history of breast or ovarian cancer.

In order to achieve the aims the following studies were initiated:

- the incidence and temporal pattern of second primary malignancies in a cohort of women with gynecological cancer (I);

- the validity and reliability of cancer registry data in dealing with second primary malignancies in women with gynecological cancer (II);

- associations between leukemia and the use of platinum-based chemotherapy in ovarian cancer patients (III);

- risk factors for second primary malignancies in ovarian cancer patients developing breast cancer (IV);

- the risk of subsequent ovarian cancer in breast cancer patients in relation to family history of breast or ovarian cancer (V).

2. SUBJECTS AND METHODS

2.1. SETTING

A tradition of high quality epidemiological studies has developed in Sweden and other Nordic countries due to features like long-time use of national registration numbers, existence of nation-wide health registries and a high public acceptance to registration. Sweden has even been appointed "a paradise for epidemiologists" (107), and international experts evaluating Swedish epidemiological research did not contradict this statement, however more moderate in their judgements, they agreed that prerequisites for epidemiological studies in Sweden are excellent (108).

With exception of study III this thesis was performed in Sweden. Study III was conducted as a multi-center study including Sweden and five other countries, Denmark, Finland, Netherlands, Canada, and the US. In all, seven centers participated. The study was supervised by the National Cancer Institute, Bethesda, MD.

2.2. DATA SOURCES

2.2.1. Personal identification

In 1947, national registration numbers were introduced in Sweden, giving every citizen a unique personal identifier (109). It was assigned to all residents alive in January 1st 1947 and has been assigned to all individuals born thereafter. The ten-digit number (nine digits at introduction) consists of six digits giving year, month, and day of birth, together with four digits making the identifier unique for each person. National registration numbers are used in all registries with very few exceptions and follow the citizens in most parts of society; including hospitals and health care. Therefore, the numbers provide an excellent possibility to link data from different sources and to minimize losses to follow-up in epidemiological studies.

2.2.2. Cancer registration

The Swedish Cancer register was established in 1958. Since reporting incident cases is mandatory for clinicians as well as pathologists and cytologists, a double notification

system was created, which by repeated examinations has shown a high degree of completeness, at present estimated to 98 % (1, 105, 106). The cancer register is regularly linked to the Cause of Death Register, and accordingly it is possible to obtain information on date and age at diagnosis together with date of death, if it has occurred. All malignancies are coded according to the International Classification of Diseases, seventh revision (ICD-7). For gynecological cancers, neither information on histological classification nor stages are available in the register, although databases including this information are developed at regional cancer registries. In-situ lesions and borderline malignancies are flagged; that is, they are possible to identify separately in the register, as are cases detected incidentally at autopsy. Concerning ovarian cancer, tumors of uncertain malignant potential, that is borderline malignancies and benign conditions like thecomas, are flagged in the register, and accordingly possible to identify. Cancer diagnoses only reported through death certificates are not recorded.

2.2.3. Cause of Death Register

The Swedish Cause of Death Register was set up in its present form in 1952, although information on causes of death has been collected since the 18th century. It includes dates of death together with main and contributory cause of death. Currently completeness of the register is estimated to be higher than 99 % (110).

2.2.4. Register of Population and Population Changes

This register is based on the 1960 census of the population of Sweden. All residents alive at the end of each year are included. Recently the registry has expanded to include also information on immigration and emigration.

2.2.5. Generation Register

The Swedish Generation Register, established in the early 1990s, provides at present information on all Swedish inhabitants born after 1931 being alive in 1960 or born thereafter. For these individuals information on close relatives, primarily parents, siblings, and children, but even aunts, uncles, cousins, etc, is possible to obtain from the register. Adoptions and other non-biological relations are flagged. Individuals who immigrated after 1960 but died or emigrated before 1992 are not included, nor are individuals immigrating after 1992.

2.3. STUDY DESIGN

Paper I

The regional cancer register of Stockholm-Gotland was used to create three cohorts of women with gynecological cancer. The register is one of seven regional cancer registers together forming the Swedish Cancer Register. It covers a region of Sweden with a mainly urban population of nearly 1.8 million inhabitants (51 % women). The women in the three cohorts were diagnosed between 1958 and 1992 with a primary malignancy of the cervix uteri, corpus uteri or ovaries. In all, 5,325 women with cervical cancer, 4,815 diagnosed with corpus cancer, and 5,060 with ovarian cancer were included in the cohorts. Follow-up started at diagnoses of the primary tumor and person-years at risk of developing second primary malignancies were ascertained up to the date of death, emigration or December 31, 1992, which ever occurred first. Observed numbers of new malignancies were compared to expected based on age- and period-specific incidence among Swedish women in order to compute standardized incidence ratios (SIR) as estimates of relative risk. To estimate the possibility of chance affecting the results, 95 % confidence intervals were computed and presented together with SIRs.

Paper II

This study was set in order to investigate the validity of cancer registration, that is, the impact of misclassifications regarding second primary malignancies. All 344 women registered with subsequent malignancies after a primary ovarian cancer were identified in the ovarian cancer cohort defined in paper I. The fact that care of gynecological cancer patients in the catchment area of the Stockholm-Gotland Cancer Register after initial surgery or other diagnostic procedures, is centralized to the clinic of gynecological oncology at Karolinska University Hospital, was helpful when tracing clinical records. In all, 334 ovarian cancer patients registered with 369 second primary malignancies were included in the analyses. Register-data was compared to histopathological diagnoses stated in the pathology records. Additionally, histopathological slides from 25 cases of ovarian cancer and 76 second primaries were randomly selected among the study-subjects for re-examination by an experienced tumor-pathologist.

Paper III

From a pooled cohort of 28,971 women with ovarian cancer identified in seven population-based cancer registries in five countries, 96 women were eligible for the study

having a verified diagnosis of leukemia and surviving at least one year after diagnosis of ovarian cancer. Each case was matched to three controls from the cohort of ovarian cancer patients. Matching criteria were register, age at diagnosis, year of diagnosis and survival without a second primary malignancy at least as long as the interval between ovarian cancer and leukemia in the case. Exposure data on chemo- and radiotherapy were abstracted from individual records. The risk related to different treatment exposures was obtained as odds ratios computed by conditional logistic regression comparing exposure history of each case with the individually matched controls.

Paper IV

From the cases of ovarian cancer and subsequent second primary malignancies identified in paper I and II, 72 women with ovarian cancer and second primary breast cancer were available for the case-control study. Three matched controls were selected from the cohort of ovarian cancer patients. Matching criteria were age at ovarian cancer diagnosis, year of diagnosis and survival without second primary malignancies longer than the interval between ovarian cancer and second primary breast cancer among the cases.

Detailed information on possible risk factors like reproductive data (age at menarche, age at menopause, parity, age at first child), and heredity, was extracted from the medical records together with data on histology of the ovarian cancer (WHO-classification) and treatment (type of treatment, doses). Information concerning family history of cancer in first degree relatives, that is, parents, siblings, and children, was primarily focused on ovarian and breast cancer, but information on any cancer incidence was also obtained. Validity of information concerning heredity was considered high regarding the appearance of cancer among first degree relatives although detailed information on site and diagnosis were often less specific, according to a previous in-department quality control (data not shown). The relative risk (RR) of developing breast cancer in relation to investigated risk factors was computed as odds ratios (OR) using conditional logistic regression analysis by the model for case control studies using matched controls, suggested by Breslow and Day (111).

Paper V

In all, 35,532 women born after 1931 and diagnosed with breast cancer between 1958 and 1998 were identified in the Swedish Cancer Register together with information on all previous and subsequent malignancies until the end of 1998. Additional follow-up data

were ascertained by linkage to the Cause of Death Register and the Register of Population Changes. After exclusion due to insufficient follow-up data, immigration and, after linkage to the Generation Register, missing notifications of relatives, 30,552 breast cancer patients were included in the study cohort being potentially at risk of developing ovarian cancer. Follow-up started at breast cancer diagnosis and ended at 31 December 1998, date of subsequent ovarian cancer, emigration or death, which ever occurred first. Expected numbers of ovarian cancers were estimated using age- and calendar specific incidence in the population. Standardized incidence ratios (SIR) were calculated by dividing observed to expected numbers of ovarian cancer. Analyses were stratified by age at diagnosis as well as occurrence of breast or ovarian cancer in relatives, which were obtained by linkage of data from the Generation Register and the Cancer Register. In the Generation Register 146,117 individuals were identified as first-degree relatives to the breast cancer patients. By linkage to the Swedish Cancer Register, information on incident breast and ovarian cancer together with date of diagnosis among the relatives were ascertained (in all, 3,689 diagnoses of breast or ovarian cancer were found among the relatives).

In order to achieve estimates of absolute risk of ovarian cancer among breast cancer patients, the annual incidence proportion was computed as the number of ovarian cancer cases divided by the number of person years. Cumulative risk of developing ovarian cancer within 30 years from breast cancer diagnosis was calculated by multiplying annual incidence by 30. The result was expressed as absolute risk in percent (AR %).

3. RESULTS

Paper I:

For cancer of the cervix mean time to SPM was twelve years, however the risk peaked during the first two years of observation, where notably high numbers of excess cases occurred in the lung, vulva and bladder. Overall, SIR was 1.58 with excess risk for cancer of the colon, rectum, lung, vulva, kidney, and bladder. After ten years or more the excess risk had decreased, but remained significantly increased at the sites listed above, except for cancer of the kidneys (Table 1).

Mean time to SPM among women with endometrial cancer was seven years with a significantly elevated overall risk of 21 % (SIR = 1.21). Excess numbers of second primary malignancies were found for cancer of the colon, ovaries, vulva, bladder, and leukemia (Table 2). As with cervical cancer excess risk was most pronounced during the first two years of follow-up (SIR = 1.79) and decreased over time. Statistically significant excess risk during the first two years of follow-up was found for breast, ovarian, and kidney cancer, but at those sites, there were no excess cases in latter follow-up periods. The risk of leukemia was most pronounced 3-9 years after the primary malignancy and remained increased even after ten years or more. An increased number of cancers of the colon, vulva, and bladder were also found in that latter period of follow-up.

In ovarian cancer patients the risk of subsequent malignancies was highest during the first two years of observation (SIR = 1.92) but contrary to cervical and endometrial cancer, the risk remained significantly increased for the entire period of follow-up. Association between ovarian cancer and subsequent leukemia was strongly pronounced and the risk estimates were highly increased during the first, as well as the second period of follow-up (SIR=17.65 and 9.23, respectively). In addition, overall incidence of second primary malignances were increased for cancers of the colon, rectum, breast, uterus, and bladder.

Table 1.
Standardized incidence ratios (SIR) with 95 % confidence intervals for sites of excess numbers of second primary malignancies in relation to time since the primary cancer diagnoses in 5.325 women with cervical cancer.

	SIR of SPM (95% CI), cervical cancer		
Site of SPM	0-2 years	3-9 years	10+ years
Colon	1.76 (0.84-3.24)	1.35 (0.72-2.31)	1.73 (1.21-2.40)
Rectum	2.08 (0.76-4.53)	1.44 (0.58-2.97)	2.34 (1.51-3.45)
Lung	3.83 (2.04-6.56)	2.82 (1.65-4.52)	1.85 (1.21-2.42)
Vulva	8.45 (3.10-18.4)	6.12 (2.47-12.7)	4.07 (1.86-7.73)
Kidney	3.20 (1.29-6.59)	1.06 (0.29-2.72)	1.56 (0.78-2.80)
Bladder	3.32 (1.33-6.84)	3.33 (1.72-5.82)	4.10 (2.81-5.79)

A notably high estimate was found for endometrial cancer in the first two years of follow up, whereas there were no excess cases during latter periods of follow up. A similar pattern was found with cancer of the kidney, that is excess numbers in the first years of follow up and lowered incidence in latter periods.

The excess risk of breast cancer decreased slowly over time, while colon cancer seemed not to follow this pattern, since the initial high risk decreased over the period from 3-9 years of follow-up, and increased again after ten years or more after ovarian cancer diagnosis.

Table 2.
Standardized incidence ratios (SIR) with 95 % confidence intervals for sites of excess numbers of second primary malignancies in relation to time since the primary cancer diagnoses in 4,815 women with endometrial cancer.

	SIR of SPM (95% CI), endometrial cancer		
Site of SPM	0-2 years	3-9 years	10+ years
Colon	**1.72** (0.98-2.79)	**1.40** (0.89-2.10)	**1.49** (1.01-2.12)
Breast	**1.64** (1.22-2.16)	**1.29** (0.99-1.65)	**0.79** (0.57-1.08)
Ovaries	9.55 (7.42-12.1)	0.44 (0.14-1.03)	0.19 (0.02-0.70)
Vulva	0.93 (0.02-5.16)	0.56 (0.01-3.10)	4.23 (1.93-8.02)
Bladder	**1.71** (0.63-3.72)	**1.13** (0.45-2.33)	**2.38** (1.41-3.76)
Leukemia	**2.22** (0.27-8.03)	**4.17** (1.68-8.59)	**2.53** (0.93-5.51)

Table 3.
Standardized incidence ratios (SIR) with 95 % confidence intervals for sites of excess numbers of second primary malignancies in relation to time since the primary cancer diagnoses in 5,060 women with ovarian cancer.

	SIR of SPM (95% CI), ovarian cancer		
Site of SPM	0-2 years	3-9 years	10+ years
Colon	**3.55** (2.14-5.55)	**1.35** (0.62-2.57)	**2.79** (1.81-4.12)
Rectum	**2.58** (1.04-5.32)	**1.50** (0.49-3.50)	**1.37** (0.50-2.99)
Breast	**1.65** (1.11-2.35)	**1.52** (1.04-2.15)	**1.12** (0.74-1.63)
Endometrium	4.74 (2.90-7.32)	1.27 (0.47-2.77)	0.97 (0.31-2.26)
Vulva	4.76 (0.98-13.9)	-	2.13 (0.26-7.69)
Bladder	**1.50** (0.31-4.38)	**2.04** (0.66-4.76)	**4.04** (2.15-6.90)
Leukemia	**17.7** (8.07-33.5)	9.23 (3.39-20.1)	-

Paper II:

The medical records of 344 women registered with ovarian cancer and subsequent second primary malignancies were scrutinized and revealed errors in cancer registration concerning both the primary ovarian cancer and the second primary malignancies. The majority of erroneous registrations could be referred to corrections of the initial cancer diagnosis due to further clinical procedures and re-examination of pathological specimens by tumor-pathologists. Although mandatory for clinicians and pathologists, the new diagnosis were not reported to the register. In all, 34 out of 344 diagnoses of ovarian cancer and 28 of 379 SPM diagnoses were incorrect. Several errors in registration of the primary ovarian cancer (68 %) concerned spurious registration of benign disorders, primary thecomas. Among 28 erroneously registered second primary malignancies, gastro-intestinal cancer were numerous, and constituted 50 % of the errors. Recalculations of standardized incidence ratios established in the previous study (Paper I) showed that the increased risk of subsequent cancer remained, however at a slightly lower level. When comparing register validity of second primary malignancies over time, the accuracy seemed to increase and was higher for the later part of the study period, 1976-1992, than for the earlier part, 1958-1975.

Paper III:

On average, second primary leukemia developed four years after the diagnosis of ovarian cancer. The results are summarized in table 4 and show a pronounced excess risk of leukemia in patients treated with platinum-based chemotherapy and even higher when this treatment was combined with other alcylating agents, primarily Melphalan. Adding radiotherapy seemed to increase the risk further.

Additionally, the association between treatment with alkylating drugs and second primary leukemia was found to be highly dependent on cumulative dose and duration of treatment with the highest risk connected to the highest doses (>1000 mg of platinum-based drugs and >250 mg of Melphalan) and longest duration (>12 months). Excess risk associated with the use of Melphalan found in the study was primarily related to the use of intravenous treatment, but investigated separately, orally administrated Melphalan was also found with an increased risk of leukemia, however of a lower magnitude.

Table 4.
Risk of leukemia, expressed as odds ratios (OR) with 95 % confidence intervals (95% CI) associated with the use of alkylating drugs and radiotherapy in treatment of ovarian cancer patients

Treatment	Leukemia cases/controls	OR	(95 % CI)
Neither alkylating drugs nor radiotherapy	5/58	**1.0**	Ref.
Radiotherapy only	1/36	**0.4**	0.04-3.5
Alkylating drug only	65/135	**6.5**	2.3-18-5
Radiotherapy + alkylating drug	25/43	**8.1**	2-6-25.6
Alkylating drugs analyzed separately:			
Platinum	27/103	**4.0**	1.4-11.4
Melphalan	28/40	**20.8**	6.3-68.3
Platinum + Melphalan	19/15	**31.5**	8.9-111.1

Paper IV:

The risk of developing breast cancer was increased, but not statistically significant, for women with heredity for breast and/or ovarian cancer (RR=1.5), while cancer heredity in general, that is, occurrence of any malignant disease among first-degree relatives, resulted in an almost doubled risk. Reproductive factors such as nulliparity (RR=1.41), high age at menopause (>51 years: RR=1.70) and high age at first childbirth (RR=1.43) were associated with increased risk of breast cancer, however without statistical significance. Potentially carcinogenic treatment with radio- or chemotherapy did not increase the risk of developing breast cancer, used neither alone nor together, while patients with mucinous ovarian cancer as well as granulosacell tumors experienced an increased risk. The findings are summarized in table 5.

Crude survival in second primary breast cancer did not differ from corresponding survival rates in primary breast cancer based on nation-wide survival rates (112).

Out of 56 breast cancer cases possible to evaluate, 43 % were found with no records of preceding symptoms, and the major part of them (17 out of 24) were diagnosed in line of routine oncological follow up. Additional four cases were captured through public screening by mammography.

Table 5.
Estimates of relative risk computed as odds ratios (OR) with 95 % confidence intervals (CI 95 %) in association with second primary breast cancer in 72 women with ovarian cancer and subsequent breast cancer in comparison with 177 matched controls in relation to suggested risk factors.

Suggested risk factors	OR	(CI 95 %)
Any family history	**1.94**	(1.01-3.72)
Family history of OC or BC	**1.50**	(0.52-4.28)
Menopause > 51 years	**1.70**	(0.76-3.81)
Nullipara	**1.41**	(0.78-2.56)
>30 at first child	**1.43**	(0.52-3.91)
Chemotherapy	**0.76**	(0.39-1.46)
Radiotherapy	**0.70**	(0.39-1.25)
Granulosacell tumor	**2.49**	(0.68-9.18)
Mucinous OC	**3.80**	(1.23-11.8)

Paper V:

The risk of subsequent ovarian cancer was found to be highly associated with both age at breast cancer diagnosis and occurrence of breast or ovarian cancer among first-degree relatives. Estimates of relative risk expressed as standardized incidence ratios and absolute risk are summarized in table 6-7. The risk was not substantially altered when restricting analysis to family history at the time of breast cancer diagnosis. Absolute risk of developing ovarian cancer before the age of 70 years was nearly seven percent in breast cancer patients younger than 40 years having a relative diagnosed with breast or ovarian cancer before 50 years of age. Additionally, despite of age, any breast cancer patient with a family history including ovarian cancer experienced a close to ten percent risk to develop ovarian cancer within 30 years from diagnosis.

Table 6.
Standardized incidence ratios (SIR) of ovarian cancer and 95% confidence intervals (CI) in 30,552 women diagnosed with breast cancer, in relation to age at breast cancer diagnosis and family history of breast and/or ovarian cancer.

	Family history (SIR with 95 % CI)			
Age at breast cancer diagnosis	No history	Any history	Breast ca.	Ovarian ca.
< 40 years	**3.3** (2.2-4.9)	**7.3** 3.1-14.3)	**5.6** (1.8-13.1)	**17.0** (1.3-50.0)
40-49 years	**1.6** (1.2-2.2)	**3.6** (2.0- 6.1)	**2.7** (1.3-5.2)	**8.7** (2.4-22.4)
> 49 years	**1.1** (0.7-1.6)	**3.9** (1.9-7.2)	**3.2** (1.3-6.6)	**6.1** (0.7-22.1)
All ages	**1.6** (1.3-2.0)	**4.3** (2.9-6.0)	**3.3** (2.0-5.1)	**9.4** (4.3-17.8)

Table 7.
Absolute risk (AR %), with 95 % confidence intervals (95 % CI), to develop ovarian cancer within 30 years after breast cancer diagnosis, computed as the annual incidence proportion times 30, in 30,552 breast cancer patients.

	Family history (AR % with 95 % CI)			
Age at breast cancer diagnosis	No history	Any history	Breast ca.	Ovarian ca.
< 40 years	**1.9** (1.2-2.7)	**3.7** 1. 7-7.6)	**2.9** (0.9-6.8)	**8.7** (1.8-25.3)
40-49 years	**1.5** (1.1-2.1)	**3.4** (1.9-5.7)	**2.5** (1.2-4.8)	**8.0** (2.2-20.5)
> 49 years	**1.4** (0.9-2.1)	**5.1** (2.7-10.4)	**4.1** (1.7-8.5)	**7.9** (0.9-28.5)
All ages	**1.6** (1.3-1.9)	**3.9** (2.7-5.5)	**3.0** (1.9-4.6)	**8.2** (3.8-15.6)

4. GENERAL DISCUSSION

4.1. METHODOLOGICAL CONSIDERATIONS

In a wide sense epidemiological studies deal with association between different factors, or determinants, and diseases, or using an epidemiological vocabulary, exposures and outcomes. To investigate these associations the epidemiologist can choose among a set of study designs. The two most widely used are cohort studies and case-control studies, which are the designs used in the studies included in this thesis.

Cohort studies

Originally, a cohort is the Roman name for a group of legionaries. In epidemiological terms, a cohort is a number of subjects, individuals, followed for a certain amount of time. Measures of exposure are made when entering the cohort and measures of outcome are made during or after the time under study. Cohort studies, sometimes referred to as follow-up studies, can be made retrospectively or prospectively. The association between exposure and the disease is most often presented as the relative risk of developing the disease followed exposure to the factor under study and calculated as the relation between the occurrence of the disease in the exposed and the unexposed. Occurrence could be measured as incidence rates, where person-years are the denominator used. It might be added that exposure could be separated into different groups or levels of exposure. Furthermore, a cohort might be closed or open, the latter meaning that it is possible for new subjects to enter the cohort during the study time and it is possible to leave before the study time has ended. The original Roman cohort was closed, that is, once established there was no recruitment of new legionaries, and the cohort existed until the last man had died.

Case-control studies

In order to be efficient, cohort studies most often need large amounts of subjects included. In a case-control design the cases should be the same individuals who would be considered cases in a hypothetical cohort study of the same population. The advantage in efficiency is accomplished by using a series of controls instead of complete assessment of exposure data in the whole cohort. Controls should be representatives for the exposure

distribution in the population in which the cases arise. There are numerous ways of selecting controls and the method used is important for validation of any associations between exposure and outcome revealed in the study. Matching cases to controls is used to avoid confounding, however to vigorous matching might be contra-productive, resulting in loss of power or even introduction of bias by over-matching. The measure of association between exposure and outcome used in case-control studies is odds ratios, which could be understood as the odds of exposure among cases in relation to the odds of exposure among controls.

Validity

Accuracy of epidemiological studies is primarily an issue of validity. External validity deals with the question if the results are applicable to other populations or patients than the ones included in the study, often referred to as generalizability. However, this consideration should not be made until the question of internal validity is answered. Internal validity is defined as absence of systematic errors (bias) and chance as explanatory factors of results presented in a study.

Bias

There are three major categories of bias: selection bias, information bias, and confounding. Selection bias is associated with how the cohort or cases and controls are recruited to the study. They should be representative for the population they are selected from. This is usually not a big concern in populations-based cohort studies, whilst in case-control studies the reproducibility of the results is entirely depending on this selection, that is, are the cases representative for the cases in the population, and, are the controls representative for the non-cases in the population?

Information bias deals with exposure measurements and classification of individuals in healthy or diseased. In cohort studies this is primarily an issue of ascertaining exposure and classifying the cohort in exposed and unexposed, but it is also important when recording outcome (diagnosis) among the cohort members. Misclassification of exposure or outcome is likely to distort the associations, however if non-differential, that is, not different and equally likely to occur in all the groups involved in the analysis, the results will be diluted and any connection between exposure and outcome will be underestimated. On the other hand, if misclassification is differential, affecting one group more than the other, the distortion of the results might take any direction. In case-control

studies this might occur as ”recall bias”, an established observation that it is more likely for cases to recall exposures in the past while controls might have difficulties remembering details on any past exposures.

Confounding is an even more complex issue in epidemiologic studies, although if possible confounders are identified and addressed in the performance of a study, confounding might be possible to adjust for in the analysis. However, if unidentified or unadjusted, a large problem emerges and confounding is likely to distort the results in any study. By definition confounding is a systematic error introduced when the exposed and unexposed subjects in a study differ with respect to other factors (confounders) that influence the risk of obtaining the outcome under investigation. The most common confounder in epidemiological studies is age. It is not difficult to imagine that for many outcomes (in this thesis, cancer) the probability is higher in elderly than in younger, and additionally, the probability of exposure might also differ between younger and older subjects. For instance, if – in an investigation of the association between nulliparity and ovarian cancer - the cases are much older than the non-cases. Then, it is most likely that ovarian cancer occur in elderly women, while nulliparity might be more likely in young women. In consequence, nulliparity could be presented as a protective factor for ovarian cancer, since the outcome ovarian cancer is less likely among the youngest. However, there are techniques available to adjust for such differences in epidemiological methodology. Stratification is the major model used, meaning that a possible confounder is stratified (divided) in to different groups (strata) and analyses of association between exposure and outcome could be performed separately for different strata, for instance, age groups.

Chance

Additionally, to accomplish internal validity the possibility of chance explaining the results must be ruled out. Statistical tests applied to the risk estimates achieved in the study is the first step in this process, providing measures of “significance”, that is, p-values or, more commonly, confidence intervals. But even then, assuming the results are “statistical significant” it should be stressed that a significance test only gives the likelihood of a result or a range of values in which it is likely that the true result is found. There is still a possibility that the true result lies outside that range. From this follows that, besides statistical tests, other considerations on the strength of the association investigated should be applied to the results, such as consistency with the hypothesis of the study,

biological credibility, dose-response relations and temporality between exposure and outcome.

Paper I

A primary methodological consideration concerning Paper I would be misclassification of outcome, that is, the second primary malignancies registered among the women with a primary gynecological cancer. For ovarian cancer this is dealt with in paper II, while for cervical and endometrial cancer the amount of misclassification is obscure. In cervical cancer, the observed excess of second primary malignancies of the lung might be misclassified metastases of the primary cancer. This consideration is most likely to be important for lung cancer occurring within 1-2 years after the primary malignancy, and accordingly the noticed association might be exaggerated. The observed increase in vulva cancer might also be due to misclassification, since cancer of the vagina and vulva were registered with the same code and cancer of the vagina might be difficult to differ from a relapse in cervical cancer. In the endometrial cancer cohort, ovarian cancer might be misclassified as a second primary malignancy, when actually being a relapse.

In this kind of cohort study the total female population serves as study base, since the outcome (different malignancies) is compared between those "exposed" to gynecological cancer and those who are not. In consequence, there are no possibilities to address the status of any association revealed in the study, since there are no data on plausible risk factors. The study could by no means be explanatory for any association revealed in the study, merely could it generate hypothesis for future studies to test.

Another limitation, and a possible confounder, in this kind of study is the "exposure", that is gynecological cancer. If the diagnosis of gynecological cancer would affect the risk of developing any second primary malignancy, comparison with the background incidence among Swedish women would be confounded. For instance, the risk of breast cancer is likely to be confounded by surgical treatment for the primary gynecological malignancy, which in most cases include oophorectomy, and is likely to diminish the risk of breast cancer. Additionally, the risk estimates of subsequent gynecological cancers are likely to be confounded by a higher prevalence of both hysterectomy and oophorectomy among gynecological cancer patients than among women in the total population.

Paper II:

This study is actually performed as a validity test of the Swedish Cancer Register, and the primary methodological concern would be a problem of external validity. For the pathologists, ovarian cancer constitutes many difficulties, perhaps most pronounced when trying to distinguish a possible relapse in ovarian cancer from a second primary cancer with similar morphological features. Especially in the early years of cancer registration, when there, beside the morphological, were no means to conclude the correct diagnosis. In consequence, relapses in ovarian cancer were, and are, difficult to identify being morphologically similar to several other cancers occurring in the abdomen. In consequence, due to these biological properties of ovarian cancer, it could be considered a "worse case" than most other primary malignancies. Therefore, it is difficult to adapt the results to other sites of primary malignancies, established with an excess risk of second primary malignancies. Neither could the findings be applied to other cancer registries with other routines and regulations. Furthermore, attention should be given the problem of true second primaries erroneously classified as relapses in the primary malignancy, in this case ovarian cancer. For obvious reasons, this issue was not addressed in the study, but would, if prominent, affect estimated associations.

Paper III:

In case-control studies a major methodological consideration could be the possibility of selection-bias. In this multi-center study of leukemia occurring in women with primary ovarian cancer extensive efforts were made in order to identify all cases of leukemia or myelodysplatic syndromes arising among the ovarian cancer patients. Not only cancer registries, where the primary ovarian cancers were detected, were searched for hematological malignancies, but also in-patient registries and in some instances regional hematological registries. In order to minimize confounding, controls were matched for age at diagnosis of ovarian cancer as well as year of diagnosis. An additional criterion was that the controls should survive without any second primary malignancy for at least as long as the interval between diagnosis of ovarian cancer and leukemia in the cases. External validity is another property of methodological consideration important to address. It is unlikely that the strong association between chemotherapy and leukemia found in this study is confounded by the indication for this treatment, that is, ovarian cancer, since there are no other suggested associations between ovarian cancer and leukemia, beside the ones investigated in the study. Therefore. In consequence, the results

appear to be valid also when applied to other malignancies, where platinum-based chemotherapy is widely used.

Paper IV:

The primary methodological concern when analyzing internal validity in this case-control study would be the role of chance affecting the results. Wide confidence intervals and weak statistical significance due to small numbers of cases could be further limited by information bias, since there are missing data for several of the analysis performed, and consequently, less power connected to any observed associations. The power would be even further diminished if information was missing for the case or all of the controls in a matched set. Then the whole set might be left out of the analysis on some exposure due to the vigorous matching. In an epidemiologist's point of view, this would serve as an example of over-matching. Another problem in performing analyses on such an amount of exposures would be the risk of mass-significance, that is, the probability of establishing a spurious association by pure chance, since the design using 95 % confidence intervals will inevitably introduce erroneous findings in one out of twenty analyses.

Furthermore, in order to properly investigate the impact of clinical surveillance, the results would benefit if information on stage of the second primary breast cancer could be added. However, such data was only available for a small number of cases but will be useful in future studies addressing this issue.

Paper V:

Strengths of the study include its population-based design, complete follow-up of all subjects in the cohort and ascertainment of exposure (family history) with optimal specificity without any differential misclassification. Among limitations to consider is some underascertainment of family history of cancer due to the design of the Swedish Generation Register. To be recorded, a relative must have been alive in 1960. Hence, relatives who died from breast or ovarian cancer before this year would be overlooked and so would any relative diagnosed before 1958. This limitation of the register might also confound comparisons over time. In addition, although this is the largest study so far, there was limited power for more detailed analyses due to small numbers of ovarian cancer cases. Extended follow-up or our cohort will remedy this concern. Lastly, the risk estimates would be biased if the prevalence of oophorectomy in the cohort differs from that in the female population used for comparison. Since oophorectomy has been part of

the treatment in young breast cancer patients, a somewhat higher prevalence of oophorectomy in the cohort seems most likely and this would only entail underestimation of the excess risk.

Further methodological considerations would be the problem of ascertaining time at risk for index cases that develop a family history during follow-up. For such an age-dependent covariate, time at risk up until date of breast or ovarian cancer in a close relative should be recorded as unexposed, and the remaining follow-up as exposed. However, such a splitting of time at risk might not be important for genetic traits that obviously do not vary over time. As a corollary, the risk estimates did not change substantially when different analytic approaches were used.

2. FINDINGS AND IMPLICATIONS

In conclusion, we have been able to establish associations between gynecological cancer and certain sites of second primary malignancies. In cervical cancer, excess numbers of second primary malignancies were established for the lung, colon, rectum, vulva, kidney and bladder. In endometrial cancer, increased incidence was revealed for leukemia and cancer of the colon, ovaries, vulva, and bladder. In ovarian cancer, excess cases were found for cancer of the colon, rectum, breast, corpus uteri, and bladder together with leukemia. When considering possible etiology of the second primary malignancies found in excess, it seems quite plausible to apply previous suggested associations, primarily, common etiology, heredity, treatment with chemo- and radiotherapy, together clinical surveillance and misclassification, some of them investigated in line of this thesis. Concerning ovarian cancer, we found that the associations - to some extent - could be explained by errors in registration, but excess risk of second primary malignancies remained even after corrections for spurious registration. Knowledge on relations between chemotherapy and subsequent leukemia has been expanded to include also platinum-based compounds and the risk of breast cancer in ovarian cancer patients seems linked to common risk factors including reproductive and hereditary factors. The latter has also been proven as a powerful predictor of ovarian cancer risk in breast cancer patients, where family history of ovarian cancer was found highly associated with subsequent ovarian cancer. However, the association needs further evaluation before implemented in clinical practice, and accordingly, as a basis for advice on regular check-ups or possible surgical interventions, such as prophylactic oophorectomy.

The clinical implications would be that established and suggested associations between first and second primary malignancies in gynecological cancer patients should be considered in clinical follow-up. Increased risk for second primary malignancies seem to be protracted over time, and therefore, the need for life-long clinical surveillance of cancer patients seems evident.

Treatment with potentially carcinogenic compounds should be considered in light of the primary effect on the initial tumor, since the positive effects of treatment by far outscores the late adverse effects in terms of second primary malignancies. Nevertheless, considerations on late effects should be included in the management of cancer patients in order to further underline the need of proper indications for any given treatment.

4.3. FUTURE ISSUES

Any future studies in the field of second primary malignancies should aim at further identify sub-groups of patients at high risk of developing new malignancies, primarily in order to increase the possibility for early detection and thus provide better prognosis.

An intriguing possibility to further address questions on family history as a risk factor for second primary malignancies is provided by the Swedish Generation Register. At present, use of the register is hampered by some systematic weaknesses, but given time to remedy this concern, the Generation Register might prove to be a valuable tool in identifying cancer patients at high risk of subsequent malignancies.

An additional field in future studies of second primary malignancies – also associated with hereditary factors - is the rapidly growing area of molecular epidemiology, where epidemiologists and molecular biologists share each others tools in order to distinguish intra-individual properties (mainly genes and gene-products) that could explain why some individuals are more susceptible to certain diseases. Development in this field is likely to increase our knowledge on etiology of second primary malignancies as well as carcinogenic mechanisms in general.

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