Non-Genomic Biomarkers of Risk in Ovarian Cancer

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Non-genomic biomarkers of risk in ovarian cancer

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1. Introduction

Cancer biomarkers are biologic substances able to measure molecular, cellular, tissue, or host factors associated with cancer susceptibility, pathogenesis, response to treatment, or survival. Most familiar are serum biomarkers associated with cancer progression like the classic tumor markers, CEA, CA 125, CA 15.3, or PSA. Their use has been extended to early detection as well as surrogate endpoints in prevention or treatment trials. Many "classic" biomarkers are secreted by cancer cells or are direct consequences of the tumor diathesis; and their levels closely correlate with tumor burden. However, other biomarkers may not depend upon a cancer being present. Rather, they may reflect risk for or susceptibility to cancer.

There are several reasons why susceptibility or risk biomarkers might represent an attractive strategy to reduce the morbidity and mortality of ovarian cancer. Risk biomarkers might point to options for chemoprevention; e.g. raising levels of a nutrient that appears to be deficient. Even if the risk biomarker suggests no obvious prevention strategy, women identified to be at very high levels of risk for ovarian cancer could be offered prophylactic surgery. Primary prevention of ovarian cancer through removal ovaries and tubes in high-

risk women is already a reality through BRCA1 and BRCA2 genetic testing, but such testing is costly and may pertain to only 10% of women who may develop ovarian cancer. Alternatively, risk biomarkers might identify women who might be suitable for more intensive screening. In this paper, we review non-genomic risk biomarkers for ovarian cancer under the following categories: micro and macronutrients, growth factors and hormones, inflammatory markers, and mucins and antibodies against them. We begin with a brief discussion of methodologic aspects affecting the discovery and application of susceptibility biomarkers for ovarian cancer.

2. Methodologic considerations

Several factors hamper the ease of investigating risk biomarkers for ovarian cancer. Case-control methodology that has been most commonly used to study ovarian cancer has the obvious limitation that the cancer cases are generally studied after diagnosis or therapy. Thus, the cancer itself may affect immune or inflammatory markers, while surgical removal of the ovaries will clearly alter levels of any hormones related to the ovarian/pituitary axis. Circulating nutrient levels are likely to be altered by the disease itself, as tumor growth may affect nutritional status, as well as by chemotherapy. Thus, study of the level of a potential biomarker many months (or years) prior to diagnosis is fundamentally important. However, the relatively low incidence of ovarian cancer (ovarian cancer is approximately 9

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times less frequent than breast cancer) makes the use of prospective study designs particularly costly and inefficient. Despite this, several cohorts are large and now mature enough so that a sufficient number of ovarian cancer cases have accumulated, making the study of risk biomarkers possible.

Another important issue to be considered is whether it is realistic to expect that a single measurement of a biomarker can capture the long-term biologic milieu in which a cancer may develop. Especially for a hormonal marker, variation by menopausal status, oral contraceptive or menopausal hormone use, phase of the menstrual cycle, or even time of day may make data from a single hormone measurement difficult to interpret. Williams and colleagues [1] showed that, aside from estrone sulfate and sex hormone binding globulin (SHBG), for which a one-time measurement captured most of the true variation in these hormone levels, a single measurement of any particular gonadotropin or sex steroid hormone is not representative of their long-term levels. Further difficulties arise from the histological diversity of ovarian cancer, where each type might be associated with distinct risk factors or susceptibility marker profiles. Finally, the etiology of ovarian cancer is not yet completely understood; thus, we don't have a clear roadmap of the biologic processes that should be targeted by potential risk biomarkers. Despite all these difficulties, several risk biomarkers have been investigated in hope they could point to a strategy to decrease the morbidity and mortality of ovarian cancer (summarized in Table 1).

3. Micro- and macronutrients

3.1. Anti-oxidants

Anti-oxidants are substances capable of reacting with or neutralizing free radicals (reactive oxygen and nitrogen species) that might otherwise cause genetic damage and promote carcinogenesis [2]. A variety of anti-oxidant micronutrients have been postulated to exert a protective effect on ovarian carcinogenesis, including carotenoids, alpha-tocopherol or vitamin E, vitamin C, and selenium. Most of the studies have focused on estimating anti-oxidant intake using dietary questionnaires. Case-control studies comparing dietary intake or supplementation between women diagnosed with ovarian cancer (prior to disease) and cancer-free women have suggested the following antioxidants may reduce risk for ovarian cancer: vitamin E [3–6], vi-

tamin C [4], vitamin A [3,7,8], carotenoids [9], beta-carotene [3,5,6,8,10–12], and lycopene [13]. However, prospective epidemiologic studies based on dietary intake failed to provide evidence to support a role of increased intake of carotenoids [14–17], vitamin A [16], vitamin C [15,16], or vitamin E [14,15] in ovarian cancer risk. A few prospective studies have evaluated serum levels of micronutrients as markers of risk. Helzlsouer et al. [18] examined the association between the levels of several micronutrients and subsequent risk of ovarian cancer and found levels of carotenoids not to be associated with ovarian cancer risk [18]. For vitamin E or alpha-tocopherol, these authors found higher levels in women who developed ovarian cancer, suggesting that greater alpha-tocopherol levels increased subsequent risk, while no statistically significant difference was observed in alpha-tocopherol levels between women who became cases and their matched controls in a small study of ovarian cancer by Knekt et al. [19].

Selenium is another anti-oxidant that may be protective against cancers as suggested in several animal models [20] and ecological data showing inverse correlations between average selenium intake or selenium blood levels and cancer mortality [21,22]. The study of selenium intake through dietary sources is not an option because selenium levels in foods vary substantially according to the selenium content in the soil. Thus several epidemiologic studies have examined levels of selenium in serum or in finger or toenails to assess selenium intake in relation to cancer occurrence, including two studies of ovarian cancer. While in one study toenail selenium levels were not found to be associated with risk of developing ovarian cancer [23], Helzlsouer et al reported greater than 70% reduction in risk for those in the highest tertile of serum selenium levels after excluding women who were diagnosed within in ovarian cancer 4 years after blood collection [18]. The discrepancy in findings is unlikely to be explained by differences in the medium in which selenium was measured in these two studies as toenail selenium seem to be highly correlated with serum selenium measurements ($r = 0.89$) [24]. Serum selenium was also not associated with risk of gynecologic malignancies in a study by Knekt et al. [25], but the number of ovarian cancer cases was not stated in this study.

3.2. Vitamin D

Vitamin D and its metabolites have been implicated in reducing the risk of several cancers, including ovarian. Some of the most commonly postulat-

Table 1
Summary of studies examining non-genetic susceptibility biomarkers in ovarian carcinogenesis prospectively

Marker	Study Design	Participants	Main Results	Assays	Reference
Micro and macro nutrients					
Serum alpha-tocopherol	Case-control nested within cohort (Finland)	16 ovarian cancer cases matched to 29 controls on municipality and age	No association with ovarian cancer risk	Intra and inter-batch CVs <10%	Knekt P. 1988 [19]
Serum α and γ-tocopherol, α and β-carotene, retinol, cryptoxanthin, lutein, lycopene, selenium, cholesterol	Case-control nested within cohort (US)	35 ovarian cancer cases matched to 67 controls on race, age, menopause, fasting, and timing of blood collection	Elevated α-tocopherol and cholesterol associated with increased risk of ovarian cancer (p-trend 0.04 and 0.02 respectively). Selenium associated with decrease in risk (p trend 0.02). Ovarian cancer risk not associated with γ-tocopherol, α and β-carotene, retinol, cryptoxanthin, lutein, lycopene.	CVs: <10% for all micro nutrients studied except for α-carotene (16.7%) and cryptoxanthin (10.8%).	Helzlsouer et al, 1996 [18]
Serum cholesterol	Prospective cohort (US)	86,464 participants, 153 ovarian cancer cases	No association with ovarian cancer risk	CVs unavailable	Hiatt and Fireman, 1986 [30]
Serum selenium	Case-control nested within cohort (Finland)	86 gynecologic cancers matched to 172 controls on municipality and age.	No association with risk of gynecologic cancers	Intra and inter-batch CVs <10%	Knekt et al, 1990 [25]
Toe nail selenium	Case-control study nested within cohort (US)	58 ovarian cancer cases matched to 58 controls on age and date of sample collection.	No association with ovarian cancer risk		Garland et al, 1995 [23]
Hormonal biomarkers					
Serum FSH, LH, androstedione, DHEA sulfate, estrone, estradiol, progesterone	Case-control study nested within cohort (US)	31 ovarian cancer cases matched to 62 controls on age, menopause status, timing of blood collection	Lower FSH associated with increased risk ($p = $ 0.04) Elevated androstedione associated with increased risk (p trend 0.008) Indication of increased risk with elevated DHEA sulfate (p trend 0.11) LH, estrone, estradiol, progesterone not association with ovarian cancer risk	Intra and inter CVs $\leqslant$10%	Helzlsouer et al, 1995 [37]
Urinary DHEA, androstedione, and aetiocholanolone	Case-control study nested within cohort (Island of Guernsey)	12 ovarian cancer cases matched to 133 controls on age and menopause status.	Lower androgens associated with increased risk (DHEA $p = $ 0.007, androstedione $p = $ 0.06). Aetiocholanone not associated with ovarian cancer risk	CVs unavailable	Cuzick et al, 1983 [42]
Serum LH	Case-control study nested within cohort (US)	58 ovarian cancer cases matched to 116 controls on age, menopause status, timing of blood collection.	No association with ovarian cancer risk	Intra and inter CVs <10%	Akhmedkhanov et al, 2001 [36]
Serum FSH	Pooled case-control studies nested in 3 cohorts (US, Sweden, Italy)	88 ovarian cancer cases matched to 168 on age and date of enrollment, cohort (all postmenopausal)	No association with ovarian cancer risk.	Intra CVs <10%.	Arslan et al, 2003 [76]
Serum testosterone, androstedione, DHEAS, SHBG, estrone	Pooled case-control studies nested in 3 cohorts (US, Sweden, Italy)	132 ovarian cancer cases matched to 168 on age, date of enrollment, menopause status, cohort)	Elevated androstedione increased ovarian cancer risk among pre-menopausal women (p trend 0.12).		

Table 1, continued

Marker	Study Design	Participants	Main Results	Assays	Reference
			Testosterone, DHEAS, SHBG, estrone not associated with ovarian cancer risk.	Intra CVs <10%, inter CVs ⩽ 15.1%	Lukanova et al, 2003 [39]
Serum IGF-1 and IGFBP-3	Pooled case-control studies nested in 3 cohorts (US, Sweden, Italy)	132 ovarian cancer cases matched to 168 controls on age, date of enrollment, menopause status, and cohort.	Increased IGF-1 levels associated with increased risk among women <55 (p trend <0.03). IGBPB-3 not associated with ovarian cancer risk.	Intra and inter batch CVs <10%.	Lukanova et al, 2002 [51]
Serum IgF-1 and IGFBP-3	Pooled case-control studies nested in EPIC cohort (Denmark, France, Germany, Greece, Italy, The Netherlands, Spain, The United Kingdom)	214 cases matched to 388 controls on study center, menopause status, age, time of blood collection, fasting status, and phase of menstrual cycle (in pre-menopausal participants)	Elevated IGF-1 associated with increased risk among women <55 years at diagnosis (p-trend 0.08) and among women who were pre-menopausal at blood collection and <55 years at diagnosis (p-trend 0.02) Elevated IGFBP-3 non-significantly associated with increased risk among women <55 years at diagnosis	Mean intrabatch CVs < 4%; mean inter-batch CVs < 13%	Peeters et al. 2007 [52]
Plasma IGF-1, IGFBP-2, IGFBP-3	Pooled nested case-control studies from 3 cohorts (US)	222 ovarian cancer cases matched to 599 controls on menopause status, and age, fasting status and PMH use at blood collection.	Suggestive of inverse association between elevated IGF-1 and cancer risk (p-trend 0.14). No significant association between IGFBP-2 or IGFBP-3 and cancer risk	Intra-assay CVs ⩽10%	Tworoger et al. 2007 [53]
Serum c-peptide, IGFBP-1, IGFBP-2	Pooled case-control studies nested in 3 cohorts (US, Sweden, Italy)	132 ovarian cancer cases matched to 168 controls on age, date of enrollment, menopause status, and cohort.	Elevated IGFBP-1 and IGFBP-2 associated with non-significant decrease in risk (among women <55 years) C-peptide not associated with ovarian cancer risk.	Intra and inter CVs ⩽11 and 16.2%	Lukanova et al, 2003 [47]
			Inflammatory markers		
Serum soluble Fas	Pooled case-control studies nested in 3 cohorts (US, Sweden, Italy)	138 ovarian cancer cases matched to 263 controls on age, date of enrollment, menopause status, and cohort.	No association with ovarian cancer risk	Intra and inter CVs <10%	Akhmedkhanov et al, 2003 [59]
			Mucins		
Serum CA 125	Case-control nested within cohort (JANUS serum bank, Norway)	105 ovarian cancer cases matched to 323 controls on age, residence, time of sample collection and sample storage conditions.	Levels of CA 125 lower among controls than cases diagnosed up to 12 years ($p < 0.001$)	Inter assay CVs <15%	Zurawski et al, 1988 [31]
Serum CA 125	Case-control study nested within cohort (US)	37 ovarian cancer cases matched to 73 controls on age and time since last menstrual period.	Levels of CA 125 lower among controls than cases diagnosed up to 12 years after blood collection ($p = 0.002$).	Intra batch CVs ranged from 6 to 16% depending on CA 125 concentrations	Helzlsouer et al, 1993 [65]
Serum CA 125	Prospective cohort (Interventional screening study)	22,000 post-menopausal women, 49 ovarian or epithelial cancer cases	CA 125 levels >30 U/ml associated with increased risk of ovarian cancer (OR and 95% CI within five years after screen: 14.3 (8.5, 24.3)	Intra and inter CVs <10%	Jacobs et al, 1996 [66]

ed mechanisms explaining the potential protective effects of vitamin D include inhibition of angiogenesis and enhancement of cell adherence, which in turn inhibit cell proliferation and may protect against tumorigenesis. Several ecologic studies have reported that ovarian cancer mortality is higher in areas of lower sunlight or lower dietary vitamin D intake (reviewed in [26]). A group of investigators have recently examined the relation between serum vitamin D metabolites (25-hydroxyvitamin D and 1,25-dihydroxyvitamin D) and risk of developing ovarian cancer in a case-control study nested within the Nurses Health Study. Overall, circulating levels of vitamin D metabolites did not seem to be associated with subsequent risk of developing ovarian cancer, although a protective effect of 25-hydroxyvitamin D was suggested among obese and overweight women ($p = 0.04$) [27], which will need to be replicated in further prospective studies.

3.3. Cholesterol

There has been some epidemiologic evidence to suggest that dietary fats, including cholesterol, may increase risk of certain cancers including ovarian malignancies [10,28]. One biologic rationale for the potential influence of high cholesterol in ovarian carcinogenesis is that cholesterol is a steroid precursor that could lead to increased biosynthesis of estrogens, which may increase ovarian cancer risk. However, in a recent pooled analysis of 12 cohort studies of dietary fats, Genkinger et al. [29] found no evidence for a role of dietary fat or cholesterol in ovarian cancer risk. Thus, it is not surprising that the association between serum cholesterol levels and subsequent risk of ovarian cancer, explored to date in only two prospective studies, produced inconclusive findings. Helzlsouer et al. [18] reported a higher than 3-fold increase in ovarian cancer risk among women in the highest tertile of serum cholesterol levels compared to those in the lowest tertile. After excluding women diagnosed within 4 years of blood collection, the ORs for those in the highest tertile remained elevated, though no longer statistically significantly elevated (OR and 95% CI: 2.9 (0.7, 11.3)). In contrast to the findings reported by Helzlsouer et al, cholesterol levels were not found to be associated with ovarian cancer incidence in a large cohort where 190 women developed ovarian cancer over the course of up to 16 years of follow-up [30].

4. Hormonal biomarkers

4.1. Gonadotropins

It has been postulated that excessive stimulation of the ovaries by pituitary gonadotropins may increase risk of malignant transformation both directly and indirectly through stimulation of sex steroid production [31]. Animal models using ovarian irradiation or chemicals toxic to oocytes raised gonadotropin levels and produced ovarian tumors, albeit not entirely comparable to the epithelial tumors in women [32,33]. Results of epidemiologic studies compatible with an effect of gonadotropin include ovarian cancers observed after irradiation for cervical cancer [34] or protection associated with birth control pill use, which profoundly lower gonadotropins [35]. The association between prediagnostic levels of gonadotropins and ovarian cancer has been examined in only two studies. In one study, no association was found [36], and in the other an inverse association was observed. In this study, Helzlsouer et al. [37] reported lower gonadotropin levels (particularly follicle-stimulating hormone) in women subsequently diagnosed with ovarian cancer compared to controls, particularly among post-menopausal women. Although relatively small and based on a single measurement of gonadotropin levels, these studies fail to provide support for a role of circulating gonadotropins as markers of increased susceptibility to the development of ovarian cancer.

4.2. Estrogens and progesterone

Estrogen and progesterone production in the premenopausal woman occurs almost entirely in the ovaries where the synthesis of sex-steroid hormones is under the control of gonadotropins acting on the granulosa and theca cells of the developing follicle. Ovarian epithelial tumors, which have the appearance of estrogen-sensitive Mullierian tissues, may contain estrogen and progesterone receptors [38]. To date, only two studies have directly investigated the relation between circulating levels of estrogens and subsequent risk of ovarian cancer [37,39]. Both studies were null, providing no support for a major role of circulating estrogens on ovarian carcinogenesis. Lukanova et al. [39] also investigated the role of sex hormone-binding protein (SHBG), which is the major binding globulin of estradiol and determines the levels of biologically active estradiol. They found no influence of SHBG on ovarian cancer risk. In this study, repeated measure-

ments of estrone and SHBG were obtained 11 to 60 months apart, and intra-class correlations of 0.89 for estrone (among post-menopausal women only) and of 0.89 for SHBG were reported.

Contrary to the presumed harmful effects of estrogen, progesterone excess has been postulated to be beneficial [40]. After the second month of pregnancy, the placental synthesis of progesterone is responsible for a close to 10-fold increase in the maternal circulating levels of progesterone, which led some to hypothesize that progesterone could account at least in part for the protective effects of pregnancy on ovarian cancer risk. However, the only study conducted to date to directly examine the association between levels of progesterone and subsequent risk of ovarian cancer found no convincing evidence for a role of progesterone on ovarian carcinogenesis [37].

4.3. Androgens

Androgens have also been proposed to increase ovarian cancer risk according to several lines of evidence reviewed by Risch [40]. Ovarian epithelial cells contain androgen receptors, and ovarian epithelial cell lines treated with androgens have shown increased proliferation rate and decreased rate of cell death [41]. Epidemiologic evidence supporting the androgen hypothesis include the protection conferred by oral contraceptive use, which reduce circulating levels of androgen and the increased risk observed with women diagnosed with Polycystic Ovarian Syndrome (PCOS), characterized by elevated circulating androgen levels.

More direct evidence for a role of androgens in the development of ovarian cancer was provided by 2 prospective studies [37,39], both of which showed an increased ovarian cancer risk (though non-significantly in one [39]) among those with elevated prediagnostic serum levels of androstenedione (particularly among pre-menopausal women). Interestingly, urinary androgens levels were shown to be lower in controls compared to cases in an earlier small prospective study conducted in the Island of Guernsey [42]. However, the small size of this study (12 cases) increases the probability that chance played a role in their findings. Alternatively, it is possible that higher urinary concentrations of androgens do not correspond to elevated circulating levels of androgens.

4.4. Insulin

Insulin has both mitogenic and anti-apoptotic properties and has been implicated in several cancers, including colon, breast, and pancreatic cancer [43]. Insulin may influence ovarian cancer risk on one hand by stimulating LH-induced androgen production, and on the other hand by downregulating the expression of sex hormone binding globulin (SHBG) and insulin-like growth (IGF) factor binding protein 1, thereby influencing levels of biologically active, free circulating sex steroids hormones and IGF-I [40,44]. Despite an appealing biologic rationale, epidemiologic data have not provided clear support for a role for insulin in ovarian carcinogenesis. Conditions intimately associated with increased insulin production such as obesity and type II diabetes mellitus have been shown to exert minimal or no effect on ovarian cancer risk, respectively [45,46]. The only prospective study examining the association between circulating c-peptide levels, a marker of pancreatic insulin secretion, also failed to provide support for a role of insulin in ovarian cancer [47].

4.5. Insulin-Like Growth Factor and its binding protein

The mitogenic and anti-apoptotic properties of IGF-I are well documented. Elevated concentrations of IGF-I are believed to play a role in the etiology of several types of cancers, including pre-menopausal breast, and prostate, lung, and colorectal cancers [48,49]. IGF-I and its binding proteins are thought to influence the regulation of ovarian follicular development and to have mitogenic and anti-apoptotic effects on ovarian epithelial cells [50]. Three prospective studies have examined the role of elevated IGF-I levels in ovarian carcinogenesis. In two studies, higher circulating IGF-I levels were associated with 2.4 to 4.7 fold increase in risk of ovarian cancer among women diagnosed at relatively young ages [52]. This increase in risk was accentuated among women who were pre-menopausal at blood collection (higher tertile associated with 5.6 fold increase in risk) [52]. Paradoxically, elevated IGF-1 levels were suggestive of a decrease in ovarian cancer risk in a recent pooled nested case-control study (p-trend non significant); their results did not appear to differ according to age at diagnosis or menopausal status [53]. Several IGF binding proteins are known to exist and function to reduce both availability of IGF-I and its biologic activity. Increasing levels of IGF binding proteins 1 and 2 were reported to exert a non-significant protective ef-

fect on subsequent ovarian cancer risk among women diagnosed before the age of 55 in a study by Lukanova et al. [49]. Although no clear relation between IGF binding protein 3 was observed in most prospective studies, Peeters et al. [52] suggested a non-significant increase in risk with increasing concentrations among women diagnosed before the age of 55 years.

5. Inflammatory biomarkers

To address some of the shortcomings of earlier epidemiologic models for ovarian cancer based on incessant ovulation or gonadotropin excess, Ness and Cottreau [54] offered their theory that inflammation might explain the role of a number of risk factors for ovarian cancer including talc use, endometriosis, and ovulation. The process of inflammation involves the production of free radicals and recruitment of inflammatory mediators such as cytokines and prostaglandins, which can be mutagenic and contribute to the development of cancer including ovarian. Several inflammatory mediators have been found to be elevated in ovarian cancer tumors and some were associated with disease progression. Cytokines including TNF-α, IL-6, IL-1α and IL-1γ were found to be elevated in ovarian tumors and in women with ovarian tumors. Levels of TNF-α were found to be indicative of ovarian tumor grade while levels of several inflammatory markers such as IL-6, IL-8, and VEGF were found to be indicative of early stage ovarian tumors [55–58].

Because presence of tumor is likely to induce inflammatory responses, it is important that studies examine the role of inflammatory biomarkers measured (preferably) several years before diagnosis. Akhmedkhanov et al. [59] examined the association between levels of soluble Fas and the risk of developing ovarian cancer in a case-control study nested within 3 cohort studies. Soluble Fas is a member of the tumor necrosis factor receptor family, is one of the major regulators of cellular apoptosis in many physiologic processes, and dysregulation of Fas-mediated apoptosis has been postulated to play a role in ovarian tumorigenesis [60]. Despite the strong biologic rational, Akhmedhanov et al. [59] found that serum soluble Fas did not differ between cases and controls failing to show that soluble Fas would be a useful susceptibility marker in ovarian carcinogenesis.

6. Mucins and antibodies against them

6.1. Epithelial mucins

The epithelial mucins are soluble or membrane-bound glycoproteins that are products of the human mucin family of genes. The first mucin to be described was MUC1, also known as CA 15.3. MUC 1 is an especially ubiquitous glycoprotein that occurs along the apical border of epithelial cells lining the genital, respiratory, and digestive tracts and breast ducts. With cancer, mucins become over-expressed and are released into the circulation and have thus become valuable tumor markers. MUC 1 has been proposed as a potential marker of for breast, pancreatic, endometrial, colon, and lung cancer. For ovarian cancer the best known mucin marker is MUC16, originally called CA 125. However, other mucins have also been studied as potential markers of ovarian carcinogenesis. Mucins MUC1, MUC2, MUC4, and MUC5AC were found to be expressed in epithelial ovarian cancer cells; MUC3 and MUC4 were associated with tumor stage and prolonged survival [61].

One of the most widely studied mucins, MUC16, or CA 125, has been approved as a marker for the monitoring of ovarian cancer, but has also been used as a potential marker for early detection. Several studies have found CA 125 to be elevated shortly prior to diagnosis of ovarian cancer [62–64]. More intriguing, however, are studies that suggest CA 125 may be elevated many years prior to cancer. In a nested case-control study, Zurawski et al. [31] showed that prediagnostic CA 125 levels as low as 10 U/ml were associated with a close to 4-fold increase in risk of developing ovarian cancer over 12 years of follow-up. In this study, median CA 125 levels were consistently higher among women who subsequently developed ovarian cancer 2, 3, or 5 years after blood collection, compared to controls. Helzlsouer et al. [65] also observed higher median CA 125 levels in cases compared to controls within 12 years of follow up, although this finding was only significant for the cases diagnosed within the first 3 years of blood collection. In this small nested case-control study, CA 125 levels as low as 10 U/ml were associated with a greater than 5-fold increase in risk of developing ovarian cancer over 12 years of follow-up. In a more recent interventional screening study of post-menopausal women, Jacobs and colleagues [66] reported that elevated blood levels of CA 125 ($\geqslant$30 U/ml) were associated with a 14-fold increase risk of developing ovarian or fallopian tube cancers within the first 5 years of CA blood
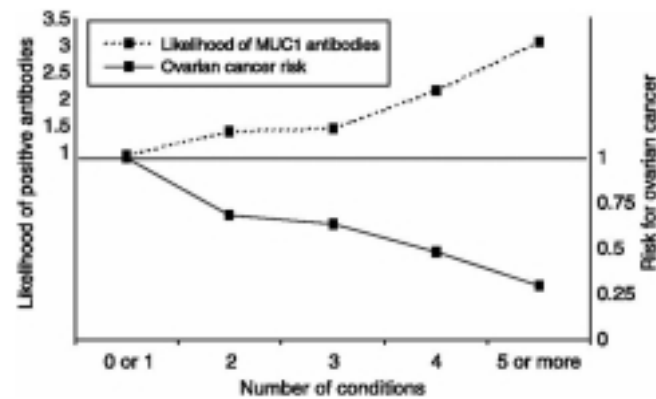
Fig. 1. Likelihood of testing positive for anti-MUC1 antibodies and risk of ovarian cancer as function of the number of protective events over a woman's lifetime (reprinted from Cramer et al. [71]).

measurements. Clearly there remains some uncertainty in these studies whether CA 125 is serving as an early detection or a risk biomarker, as some women who developed ovarian cancer shortly after blood collection were included in these analyses. However, the fact that CA 125 levels were noted to be higher in women who developed ovarian cancer 4 to 12 years after blood collection compared to those who remained cancer-free, suggests a role for CA 125 as a marker of susceptibility for ovarian cancer [65] in addition to its more recognized role as a recurrence biomarker. Further and larger prospective studies in which blood levels are measured a decade or more before the development of the cancer may be necessary to help us better define the role of CA 125 levels in ovarian cancer susceptibility.

6.2. Anti-MUC1 antibodies

An epidemiologic basis for proposing immunity as a basis for interpreting cancer risk probably dates back to 1980 with the "fetal antigen" theory for breast cancer [67]. This theory proposed that, during a pregnancy, women are exposed to fetal antigens that may be similar to antigens expressed by breast tumors and form an immunity to them that may account, at least in part, for the long-term protective effect of pregnancy on breast cancer risk. Later it was proposed that the cell surface glycoprotein and human mucin (MUC) family member, MUC1, might be the hypothetical immunity-inducing antigen since it is both secreted during pregnancy and over expressed in breast cancer [68]. It has been shown that sera from multiparous women – but not from nulliparous women or from men – are able to mediate killing of breast cancer cells. Supporting a key role for MUC1 in these reactions, core-peptide sequences from

MUC1 can induce proliferation of T cells and cytotoxic T cell responses in multiparous women [69]. The "fetal antigen" hypothesis was extended to ovarian cancer after it was shown that sera from multiparous women also reacted with multiple antigens from ovarian cancer cells more strongly than sera from nulliparous women or men [70], although MUC1 was not specifically examined in these experiments.

Because it is known that many cancers, including ovarian, over-express epithelial mucin, MUC1, and promote anti-MUC1 antibodies that correlate with more favorable prognosis, it was recently proposed that risk for ovarian cancer might be reduced by pre-existing MUC-1 specific immunity [71]. In this paper, anti-MUC1 antibodies were measured in 705 control women, and events were identified that predicted antibodies. Ovarian cancer risk was then estimated by comparing profiles of events generating antibodies in controls with those in 668 ovarian cancer cases. Factors predicting antibodies included oral contraceptive use, breast mastitis, bone fracture or osteoporosis, pelvic surgeries, non-use of talc in genital hygiene, and to a lesser extent intrauterine device use and current smoking. There was a significant increase in the likelihood of having anti-MUC1 antibodies from 24.2% in women with 0 or 1 condition, to 51.4% in those with 5 or more conditions. By the same index of events, the risk for ovarian cancer was inversely associated with number of conditions predisposing to anti-MUC1 antibodies. Compared to having experienced 0 or 1 event, the adjusted risk for ovarian cancer decreased progressively with RRs (and 95% CI) of 0.69 (0.52, 0,92), 0.64 (0.47, 0.88), 0.49 (0.34, 0.72), and 0.31 (0.16, 0.61), respectively for women with 2, 3, 4, and 5 or more events related to the presence of antibodies (p for trend

< 0.0001) (Fig. 1). The authors concluded that several traditional and new risk factors for ovarian cancer may be explained by their ability to induce MUC1 immunity through exposure of MUC1 to immune recognition in the context of inflammatory or hormonal processes in various MUC1-positive tissues.

Although intriguing, it should be appreciated that the role of anti-MUC1 antibodies as risk biomarkers was not directly assessed in this case-control study but rather only inferred by comparing the frequency of events predicting antibodies in cases and controls. Thus the value of anti-MUC1 antibodies as a risk biomarker still needs to be assessed in pre-diagnostic sera. In addition, other immune antibodies to ovarian cancer antigens might also be useful as risk biomarkers including, for example, antibodies against MUC16.

7. Discussion

During the last decade, there has been an increased interest in the use of biomarkers in cancer research to identify early disease and better understand the acquired and inherited susceptibility to the development of cancer. Our review has focused on non-genomic risk biomarkers including nutrients, hormones, and inflammatory or immune factors. Despite a large number of case-control studies based on dietary estimates of nutrient intake, prospective studies of serum nutrient levels have not generally shown a good correlation with ovarian cancer risk. Among the micronutrients studied, it is possible that elevated selenium levels may indicate lower ovarian cancer risk and elevated vitamin E and cholesterol levels may indicate some increase in risk. However, inconsistencies in the studies and uncertain biological mechanisms connecting nutrients with ovarian carcinogenesis suggest the need for further prospective studies before a definitive chemopreventive plan involving nutrients is suggested, even though this may have popular appeal.

Regarding hormonal biomarkers of risk for ovarian cancer, we found little evidence to support a major role for estrogens, progesterone, SHBG, and insulin, in ovarian carcinogenesis. On the other hand, there is some evidence suggesting that increased circulating levels of IGF-I and androgens may indicate an increased risk of ovarian tumors, especially among pre-menopausal women. Although it is not yet certain how the latter two hormones might operate to increase ovarian cancer risk, it has been observed that androgens may synergize with IGF-I to increase proliferation

of cancer cell lines [72,73]. From a chemoprevention standpoint the observation regarding IGF-I and Androgens are important since oral contraceptives are able to significantly lower both androgens and IGF-I [74, 75]. This provides an additional rationale for use of oral contraceptives to lower ovarian cancer risk in addition to its ability to reduce the damage from "incessant ovulation."

It is now generally accepted that inflammatory/immune factors may play important role in carcinogenesis including that related to the ovary. Although larger prospective studies that exclude women diagnosed within a couple of years of blood collection will be needed before definitive conclusions can be drawn, antibodies against MUC1 may prove to be a promising marker indicative of ovarian cancer risk. A better understanding of the role of MUC1 antibodies in ovarian carcinogenesis will provide greater insight into the etiology of this malignancy and may point towards novel treatment and prevention strategies against ovarian cancer.

In conclusion, at the present time, there does not seem to be enough evidence to suggest any biomarker with a strong enough impact on ovarian cancer risk to justify recommendations of preventive interventions such as the removal of ovaries or fallopian tubes. Rather, this review points to a few markers that may have important implications in chemoprevention or in the targeting women for more intensive screening.

Acknowledgements

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