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Serum immunoglobulin levels and the risk of bladder cancer in the AMORIS Cohort

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Abstract

Background The anti-tumour T-cell response in bladder cancer has been shown to correlate with response to treatment and prognosis. However, little is known about the role of humoral immunity in this highly immunogenic human cancer, which is characterised by a high mutation-associated neoantigen load and a strong response to immunotherapy. In the present study, we interrogated the Swedish Apolipoprotein Mortality Risk Study (AMORIS) to explore the relationship between pre-diagnostic serum immunoglobulin levels and the risk of developing bladder cancer.

Methods Our analysis included all AMORIS participants aged 20 years or older, who had all three major serum immunoglobulins (IgA, IgM, IgG) recorded at the same baseline measurement (n=29,876). All participants were free from bladder cancer at the time of measurement. Samples were obtained between 1985-1996, with follow-up information until 2011. Multivariate Cox proportional hazards regression was used to investigate the association between bladder cancer risk and different levels of pre-diagnostic serum immunoglobulins.

Results: During a mean follow-up period of 15.31 years, 163 (0.5%) individuals were diagnosed with bladder cancer. Multivariate Cox regression showed an inverse association between pre-diagnostic serum IgM levels ≥ 1.4 g/L and bladder cancer risk compared to serum IgM levels < 1.4 g/L [HR: 0.68 (95% CI 0.45-1.03)]. Corresponding associations could not be established for serum IgA or IgG.

Conclusion: Our findings implicate serum IgM in the pathogenesis of bladder cancer and suggest that the concept of "humoral immune surveillance" against cancer warrants further mechanistic investigation.

INTRODUCTION

Cancer of the urinary bladder is the ninth most common type of cancer worldwide and is responsible for an estimated 165,000 deaths annually (1). Bladder cancer encompasses two main disease entities, which are characterised by a disparate clinical course, response to treatment and prognosis. Non-muscle invasive bladder cancer (NMIBC) accounts for 75% of cases at diagnosis (2). The mainstay treatment of NMIBC consists of transurethral resection of bladder tumour (TURBT), followed by intravesical chemotherapy or intravesical Bacillus Calmette Guérin (BCG) therapy for intermediate- or high-risk tumours (3). The remaining 25% of cases present with organ-confined muscle-invasive bladder cancer (MIBC) or metastatic disease and have a significantly worse prognosis (4).

A first indicator of the strong immunogenicity of bladder cancer was provided in 1990 following the approval by the US Food and Drug Administration of intravesical BCG for NMIBC (5). Despite being the first licenced form of active cancer immunotherapy, its exact mechanism of action is still incompletely understood (6). The seminal work of Schreiber et al. on cancer immunoediting in early 2000s allowed us to appreciate the central and multifaceted role of the immune system in carcinogenesis and paved the way for fundamental advances in the field of cancer immunology within the following decade(7). In parallel, the development of immune-checkpoint inhibitors revolutionised the treatment of a rapidly increasing number of solid tumours, and bladder cancer constitutes a prime example. Since 2014, accumulating evidence supporting the effectiveness of immune checkpoint blockade in advanced bladder cancer has paved the way for the entry of five such agents into routine clinical practice (8).

In contrast to the well characterised role of T-cells in mediating cancer immunosurveillance, the contribution of humoral immunity in sculpturing the evolution of tumour is far less investigated (9). Still, the ability of the humoral immune system to identify and destroy transformed cells has been supported by both clinical and laboratory evidence (10). Firstly, spontaneous antibody responses in cancer patients have been reported for more than 100 different tumour-associated antigens (11). Secondly, paraneoplastic syndromes, cancer-related disorders in which manifestations in distant organs are mediated by humoral factors rather than by direct tumour invasion of surrounding tissue or metastasis, have been associated with a wide-range of auto-antibodies directed against self-antigens (12). In paraneoplastic neurological syndromes, anti-tumour antibodies can be detected years before the clinical diagnosis of the underlying malignancy and often disappear following tumour removal, a finding that implicates humoral immunity in the immunosurveillance of incipient tumours (13). More recently, tumour infiltration by B cells has been shown to be associated with favourable outcomes in different malignancies (14, 15). Our previous work has explored the association of serum IgE levels with the risk of developing different types of cancer (16), as well as the role of IgG4 in mediating immune escape and the association with worse clinical outcomes in melanoma (17).

The anti-tumour T-cell response in bladder cancer, similar to other malignancies, has been shown to be an important determinant of response to treatment and prognosis (18). However, little is known about the role of humoral immunity in this highly immunogenic human cancer. In the present study, we interrogated the Swedish Apolipoprotein Mortality Risk Study (AMORIS) to explore the relationship between pre-diagnostic serum immunoglobulin levels and the risk of developing bladder cancer.

METHODS

The AMORIS study population

The AMORIS prospective cohort study contains data on 812,073 Swedish men (49%) and women (51%) included between 1985 and 1996, with follow-up information on incident cancer and death extended up to 2011 (19). Participants were either healthy individuals having blood and urine samples taken as part of a yearly routine check-up and occupation health screening or outpatients referred for clinical laboratory testing. None of the participants was a hospital inpatient at the time of testing and none was excluded due to disease manifestations or treatment. The age, gender and socioeconomic distribution of participants were similar to that of the general population in the Greater Stockholm County. The mean age at the time of the first investigation was 42.6 years (20). Laboratory analysis of all samples was performed at the Central Automation Laboratory (CALAB) in Stockholm, Sweden. The unique national identification number of each participant has allowed the linkage of the CALAB data with multiple Swedish national registries, including the National Cancer Register, the National Cause-of-Death Register, the National Patient Register and the Swedish Censuses (1970-1990). This has enabled the study of a plethora of clinical and socio-demographic information, such as cancer diagnosis, cause-specific mortality, comorbidities, socioeconomic status, education level and emigration.

For the current study, we included all participants aged 20 years or older who had the three major serum immunoglobulin levels (IgA, IgM and IgG) recorded at the same baseline measurement (n=29,876). All participants were free from bladder cancer at the time of blood sampling, as registered in the National Cancer Register. Incident cases of bladder cancer were identified from the Swedish National Cancer Register using the International Classification of Diseases (ICD-7: 181). The National Cancer Register is mandatory for reporting of diagnosed cases of cancer in Sweden and goes back to 1958. The National Cause of Death Register includes all deaths in the population of Sweden with a very high degree of completeness. Follow-up time was defined as the time from baseline measurements until the date of bladder cancer diagnosis, death from any cause, emigration or the end of study (31st of

December 2011), whichever occurred first. Co-morbidities were identified from hospitalization data recorded in the National Patient Register and assessed by calculating the Charlson Co-morbidity Index (CCI), as previously described (19). The National Patient Register has a complete national coverage of in-hospital patients from 1987 (regionally from 1964) and of specialized out-patient care since 2002. The level of education was categorised into low (primary school or less), middle (high school) and high (higher education). Age at baseline blood test was obtained from the CALAB database.

Serum total IgA, IgM, IgG (g/L) were measured by turbidimetric determination with reagents from DAKO (Glostrup, Denmark) using Hitachi 911 automatic analyser (Boehringer-Mannheim, Germany) with a coefficient of variation < 5 % (IgA), ≤ 5 % (IgG) and ≤ 7 % (IgM).

Data Analysis

Multivariate Cox proportional hazards regression was used to investigate the association between bladder cancer risk and different levels of pre-diagnostic serum IgA (< 0.70, 0.70-3.65, ≥ 3.66 g/L), IgG (< 6.10, 6.10-14.99, ≥ 15.00 g/L) and IgM (< 1.40, ≥ 1.40 g/L). Medical cut-offs, in the first instance, were based on values obtained from the Central Automation Laboratory (CALAB) in Stockholm, Sweden and were consistent with previously published studies (21). Due to a low number of participants with serum IgM >2.3 g/L (upper limit of normal range according to CALAB data and Gonzalez et al. (21), we instead dichotomised data based on the medical cut-off for IgM of 1.4g/L according to previously published guidelines (22).

The models were adjusted for age as a continuous variable, as well as gender, CCI and level of education as categorical variables. A test for trend was performed by using assignment to categories as an ordinal scale. To assess reverse causation, a sensitivity analysis was conducted in which those who had a follow-up time < 1 year and < 3 years were removed. Statistical analysis was performed using the IBM SPSS statistical software, Version 25.0 (Armonk, NY: IBM corp.).

RESULTS

The characteristics of the study population by bladder cancer diagnosis, as registered in the National Cancer Register, are shown in *Table 1*. More than 60% of the study population were female (63.6%). During a mean follow-up period of 15.31 years, 163 (0.5%) individuals were diagnosed with bladder cancer. The mean age at the time of

blood sample collection was 61.4 years for participants who were subsequently diagnosed with bladder cancer, compared to 50.7 years for individuals without bladder cancer.

Multivariate Cox proportional hazards regression was used to investigate the associations between bladder cancer risk and different levels of serum IgA (< 0.70, 0.70-3.65, ≥ 3.66 g/L), IgG (< 6.10, 6.10-14.99, ≥ 15.00 g/L) and IgM (< 1.40, ≥ 1.40 g/L) (Table 2). Analysis showed a lack of clear association between serum total IgA levels and bladder cancer risk. For serum total IgG, a weak inverse trend was noted, albeit not statistically significant [HR 0.83 (95% 0.50-1.39)]. An inverse association was observed between serum IgM levels ≥ 1.4 g/L and bladder cancer risk compared to serum IgM levels < 1.4 g/L [HR: 0.68 (95% CI 0.45-1.03)]. A sensitivity analysis to assess reverse causation, performed by excluding participants with follow-up time less than 1 and 3 years, showed a similar pattern for serum IgM (results not shown).

DISCUSSION

In this study, we investigated the relationship between pre-diagnostic levels of the major serum immunoglobulins and the risk of developing bladder cancer. In the AMORIS cohort we observed an inverse association between pre-diagnostic serum IgM levels and the risk of developing bladder cancer. These findings point to protective roles for components of the humoral immune surveillance. For serum total IgA or IgG levels we could not establish corresponding associations, but in some comparisons the number of exposed cases was low.

Only a few observational studies to-date have investigated serum immunoglobulin levels in patients with solid cancer. Higher levels of serum IgA have been reported in patients with head and neck cancer compared to controls (23), while a higher IgA level has been associated with more advanced disease stage (24). More recently, a lower level of total serum IgG, as well as lower levels of IgG1 subclass antibodies, were reported to correlate with poor prognosis in gastric cancer patients (25). Our group has previously reported dysregulated levels of serum IgG4, the least abundant (<5%) subclass of IgG, in melanoma patients compared to controls and its association with worse clinical outcome (17). These reports underpin the hypothesis that different immunoglobulin isotypes, as well as individual subclasses, could play diverse and contrasting roles in the immunosurveillance and immunoediting of different types of cancer. We have previously investigated the relationship between pre-diagnostic serum IgE levels with the risk of developing common cancers (26).To our knowledge, however, there are no previously published studies investigating the relationship between *pre-diagnostic* levels of the major serum immunoglobulin levels (IgM, IgA and IgG) and the risk of developing solid tumours.

IgM is the main component of an immunoglobulin subset known as 'natural antibodies'. These are *germline* encoded immunoglobulins that are produced by the human equivalents of murine B-1 B-cells (27). The natural IgM repertoire appears to be largely independent of exogenous antigen stimulation and is directed mainly against self-antigens (28)(28)(28)(28). Natural IgM has diverse functional roles ranging from immune regulation (29) to cross-reactivity with evolutionary conserved pathogen-associated epitopes (30), as well as cancer immunosurveillance (31). Primarily circulating as a pentamer and thus possessing high *avidity*, natural IgM is ideally suited to participate in cancer immune surveillance by binding to tumour-associated antigens that are selectively overexpressed in transformed cells, potentially at early stages of disease presentation (32, 33). A low *affinity* against self-antigens is also in favour of the escape of natural IgM from classic immune tolerance mechanisms, while the risk of autoimmunity is negated (34). Polyreactive natural IgM antibodies can mediate the destruction of transformed cells by a plethora of mechanisms, such as complement-mediated cell lysis, induction of apoptosis (35) and initiation of adaptive immunity against *neoantigens* (33). However, the fact that natural IgM is germline-encoded might restrict its protective effect only against cancers with high clonal neoantigen load, which have immunogenic mutations established early in tumour evolution (36). A similar mechanism has been shown to govern cancer immune surveillance mediated by T-cells (37).

Human studies have shown that natural IgM antibodies against tumour-associated antigens can be detected in the serum of both cancer patients and healthy donors (38) and many are already present at birth (39). Hernandez et al. recently reported that B-1 cells from non-small cell lung cancer patients exhibit reduced ability to produce natural IgM against a common tumour-associated antigen compared to B-1 cells of age-matched controls (40). The authors observed a similar decrease in the function of healthy donor B-1 cells with increasing age, as previously reported in animal studies (41). In a recent study utilizing plasma proteasome maps, certain forms of IgM were found to be significantly lower in patients with bladder cancer compared to controls (42).An important merit of our study is the *prospectively* acquired measurement of serum immunoglobulin levels in the AMORIS cohort, which have all been performed using the same method in a single laboratory. Moreover, Cox proportional hazard models were adjusted for several well-known confounding factors. At the same time, however, our study was limited by lack of patient information on smoking status and body mass index (BMI). In addition, data was not available on the specific type of bladder cancer diagnosed for each patient, which would enrich the implications of our findings for a better understanding of bladder cancer immunology. Finally, the low proportion of participants with all three immunoglobulin levels recorded (29,876 out of 812,073 individuals included in the AMORIS cohort) in combination with the fact that bladder cancer is a relatively rare outcome in the primarily young and healthy AMORIS cohort resulted in only 163 cases available for analysis. "As a result, further investigations, such as stratification of bladder cancer risk by gender, were inconclusive due to small numbers and results should be interpreted with caution. While

women have a markedly reduced incidence of bladder cancer, they usually present with more aggressive disease even after accounting for disease stage, which might explain the inferior clinical response to BCG in women with NMIBC, as well as worse outcomes after cystectomy for MIBC(43). Evidence suggests that women possess a more efficient humoral immune system compared to men, as reflected by a higher number of B cells, basal serum IgM level and antibody response to vaccination (21, 44). Whether the more aggressive nature of bladder tumours in females is the product of more extensive immunoediting by the humoral immune system remains to be investigated in future studies (45). Furthermore, associations of serum immunoglobulins with the development of non-muscle-invasive or muscle-invasive types of bladder cancer, or associations with disease outcomes in patients with bladder tumours remain to be thoroughly investigated(46).

In summary, our analysis of the AMORIS cohort suggests that serum IgM levels may play an important role in the immunosurveillance of bladder cancer. Further prospective studies are needed to systematically investigate the association of serum immunoglobulin levels with different types of solid cancers, their association with clinical outcomes, as well as response to treatment.

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Conflicts of Interest

The authors have declared that no conflict of interest exists.

Ethical Approval

The study complied with the Declaration of Helsinki and was approved by the ethics review board of the Karolinska Institute.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

Authors contributions

IP, SK, SS, MVH and NH were involved in the study design. Data analysis and interpretation was performed by IP, SS, MVH, NH and GW. The first draft of the manuscript was written by IP. The final version of the manuscript was edited by SK, SS, MVH, DJ, NH, GW and IP.

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