Postnatal risk factors for testicular cancer: The EPSAM case-control study

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Abstract

Testicular cancer is considered to originate from an impaired differentiation of fetal germ cells, but puberty could represent another time window of susceptibility. Our study aimed at investigating the association between environmental exposures acting during puberty/adolescence (13–19 years of age) and the risk of testicular cancer. We used data of the EPSAM study, a case–control study on germ-cell testicular cancer conducted in the province of Turin, Italy, involving cases diagnosed between 1997 and 2008. Histologically confirmed cases ($n = 255$) and controls ($n = 459$) completed a postal questionnaire focusing in particular on the pubertal period (namely age 13 years) with questions on physical activity (competitive sports, gardening), lifestyle (alcohol consumption, smoking), occupational history and medical conditions. All analyses were adjusted for the matching variables, cryptorchidism and educational level. Having done at least one competitive sport during puberty (odds ratio [OR]: 0.72, 95% confidence interval: 0.52–1.00), gardening activities during puberty (OR: 0.62, 0.42–0.94) and having a lower weight than peers during puberty (OR: 0.64, 0.42–0.97) were all inversely associated with the risk of testicular cancer. No evidence of association between smoking or alcohol consumption during puberty and the risk of testicular cancer was observed. Regarding agriculture-related occupations, we found an association with the risk of testicular cancer both for occasional jobs during puberty (OR: 2.40, 95% CI: 1.08–5.29) and ever employment in adolescence (OR: 2.59, 95% CI: 0.83–8.10). Our results suggest that postnatal exposures could play a role in testicular cancer aetiology, at least when acting in puberty or adolescence.

Abbreviations

- CI
    - confidence interval
- ISCO-88
    - International Standard Classification of Occupation
- OR
    - odd ratio

Introduction

Testicular cancer is the most common malignancy among young men in Europe and North America [1]. Approximately 95% of testicular tumours are germ cell cancers, which are histologically classified into seminomas and non-seminomas. The incidence of testicular cancer has been increasing over the past decades in many countries [2] for unknown reasons, and this increasing trend cannot be explained by established risk factors of the disease (age, ethnicity, area of residence, cryptorchidism and contralateral testicular cancer) [3].

Testicular cancer peaks at 30 years of age and evidence suggests that exposures acting *in utero* and in the perinatal life are likely to be important in its aetiology [4, 5]. However, postnatal factors, especially exposures acting during puberty, may also play a role in testicular cancer developmen [3, 6, 7]. For example, the increased risk of testicular cancer associated with cryptorchidism seems to be much larger if cryptorchidism is not treated before puberty [8]. Puberty, starting in males at an average age of 13 years, is a period of high-cellular replication, and spermatogonia, which remain inactive during childhood, start undergoing meiosis to form spermatocytes under the stimulation of sex hormones. Late age at puberty has been found to be inversely associated with the risk of testicular cancer [9].

We conducted a case–control study in Italy with the aim of exploring postnatal exposures in association with the risk of testicular cancer, with specific interest in exposures occurring around pubertal age. In particular we focused on physical activity, lifestyle, occupations, agriculture-related exposures and medical conditions.

Methods

Study design

The EPSAM study is a population-based case–control study on germ-cell testicular cancer diagnosed between 1997 and 2008 among residents of the province of Turin, Italy. Full details of our study design have been described elsewhere [7, 10]. The study was approved by the local Ethical Committee and informed consent was obtained from each subject.

We identified cases from two sources to cover the entire population of the province of Turin, and all cases were listed in the Hospital Discharge Registry of the Piedmont Region. The first source of cases included inhabitants of the province of Turin who were diagnosed with testicular cancer between 1997 and 2008 and were patients at the San Giovanni Battista Hospital (the main hospital in the city of Turin). The second source involved general practitioners (GPs) of the province of Turin, whose list of patients included at least one patient with a history of orchiectomy (ICD-9 CM surgical procedure codes: 623–624) performed for testicular cancer (ICD-9 CM diagnostic code: 186) between 1997 and 2008. In total 81% ($n = 345$) of the identified GPs agreed to collaborate and invite the cases to participate in our study. All cases were resident in the province of Turin, but they were contacted through their oncologist if they were patients at the San Giovanni Battista Hospital, or through their GP if they were not.

Confirmation of the diagnosis was provided by the histological reports. The study was restricted to germ-cell testicular cancers: 14 cases were excluded as they had a tumour of non-germ cell origin, a spermatocytic seminoma or a cancer *in situ*. One case with missing histological information was included in our study assuming that the tumour was most likely of germ-cell origin.

Controls were also resident in the province of Turin and were selected from the same two sources used to identify the cases. For each case selected from the San Giovanni Battista Hospital, we selected two patients admitted in different wards of the same Hospital between 2008 and 2009 for non-neoplastic diseases unrelated to hormonal factors and infertility, frequency matched to cases on year of birth and residence. In particular, patients were recruited at the ENT (ear nose throat) wards (47%), urology wards (24%), mainly including patients who underwent circumcision, lithotripsy unit (11%), blood bank outpatient ward (8%), gastroenterology outpatient ward (6%) and other wards of the hospital (4%). For each of the cases identified through their GP, we randomly chose two controls from the GP lists, matching for year of birth and GP.

Between 2008 and 2010, a postal questionnaire was sent to cases and controls and a saliva sample was collect from the participants, implying that cases and controls were contacted and interviewed during the same time period. The overall response rate was 57% among cases and 48% among controls. The response rate was higher among the subjects contacted through their hospital doctor (82% for cases and 84% for control) than among the patients contacted through their GP (49% for cases and 40% for controls).

Exposure assessment

The questionnaire focused mainly on exposures acting during puberty, as well as on the main established or suggested risk factors for testicular cancer. Age of 13 years was used arbitrarily as the reference age for the pubertal period. Questions concerned physical exercise, lifestyle, anthropometrical characteristics, occupations (occasional or professional), agriculture-related exposures and medical conditions.

Regarding physical activity, participants were asked to mention whether they had practiced competitive sports at age 13 years and, in case, to specify the type of sport using a free text. We then grouped a-posteriori the competitive sports following the classification suggested by Diehl *et al*. [11], which is based on the physical and technical demand of each activity. The questionnaire also asked the subjects to report the frequency, if any, of gardening activities at age 13 years. Gardening not only is a proxy of physical activity but also a possible source of exposure to pesticides. Anthropometrical characteristics were assessed by asking cases and controls to report their weight and height at age 13 years compared to peers (lower, same, higher than peers).

Information on lifestyles focused on alcohol intake and active and passive tobacco exposure at age 13 years. The questionnaire recorded the full occupational history (for each occupational period that lasted at least 1 year: age at start and end, job title and branch of industry), but for our study we focused only on jobs held between 13 and 19 years of age. The questionnaire included also questions on occasional jobs (free text) carried out at age 13 years. Job periods and occasional jobs were coded blindly to case–control status by a trained coder, according to the 1988 version of the International Standard Classification of Occupation [12]. This classification is based on 4 digits and increases specificity with increasing number of digits. As we were specifically interested in occupational exposures occurring in the agricultural setting, we created a category grouping all men who had ever worked in at least one of the following ISCO 1988 codes: 1311 – Manager in agriculture, forestry and fishing; 611 – Market gardeners and crop growers; 921 – Agricultural, fishery and related Labourers. In the occupational section of the questionnaire, participants were also asked to report whether they had ever been occupationally exposed to pesticides, including age at start and end of the exposure.

Finally, the questionnaire included questions on early medical history, asking the participants to report if they had ever been hospitalized up to 18 years of age and, in case, to report the cause of each hospitalization (up to nine hospitalizations) in free text. We then grouped the reasons of the hospitalization using an a-priori classification based on the expected frequency of the hospitalizations up to age 18 years (e.g., appendectomy) and previous evidence of association with the risk of testicular cancer (e.g., genital malformations). Hospitalizations due to cryptorchidism were excluded. However, since the EPSAM questionnaire asked questions on whether cryptorchidism was still present at age 15 years (i.e., after puberty) or not, we analysed this variable to identify men whose cryptorchidism had resolved spontaneously, medically or surgically before puberty.

Statistical methods

We used unconditional logistic regression to estimate odd ratios (ORs) and 95% confidence intervals (CIs) of testicular cancer, adjusting for the matching variables, namely year of birth (in 5-year groups), area of residence (city of Turin or the rest of province of Turin) and method of contact/identification of the study subject (GPs or hospital). We broke the individual matching, as, due to the low response proportion, using conditional logistic regression would have resulted in a large number of incomplete strata and a consequent severe loss of study power. All analyses were also adjusted for age at diagnosis for cases and a comparable reference age for controls (randomly assigned on the basis of the cases' age-distribution), educational level (junior high school or less, high school, university degree) and cryptorchidism (self-reported as having been confirmed by a physician). The analyses on agriculture-related exposures were additionally adjusted for area of residence at age 13 years (self-reported: urban, semi-urban, rural area) and for having carried out gardening at age 13 years, as employment or occasional work in the agriculture industry is expected to be associated with rural residence and gardening. Likewise, the analyses on weight were additionally adjusted for height.

Occasional jobs carried out at age 13 years and occupations carried out before age 19 years were analysed separately. Apart from analysing agriculture-related jobs as a-priori exposures of interest, we conducted an exploratory analysis on 2-, 3- and 4-digit ISCO-88 codes including at least 10 exposed subjects.

For exposure variables with quantitative information, we conducted dose–response analyses, including monthly frequency of gardening (<4 times, 4–7 times, >7 times) and years of occupational exposure to pesticides.

For analyses on occupations and on exposure to pesticides, which were both assessed up to 19 years of age, we introduced a lag time of at least 3 years between the occurrence of the exposure and diagnosis of testicular cancer

Analyses were performed using the software STATA 12 (StataCorp LP, College Station, TX).

Results

Individuals not born in Italy (5 cases and 6 controls) and 2 controls with missing information on educational level were excluded from our study, leaving, in total, 255 testicular cancer cases (54% seminomas and 46% non-seminomas) and 459 controls for the analysis.

The distributions of selected characteristics of our study subjects are reported in Table 1. Prevalence of cryptorchidism, as expected, was higher in cases (11.4%) than in controls (3.0%), while educational level was similarly distributed among cases and controls.

Table 1. Selected characteristics of case and control subjects

	Cases *N* = 255		**Controls *N* = 459**	
Characteristic	***N***	**(%)**	***N***	**(%)**
Year of birth				
1955–1964	82	32.1	168	36.6
1965–1974	100	39.2	165	35.9
1975+	73	28.6	126	27.5
Method of recruitment				
General practitioners	170	66.7	308	67.1

	Cases *N* = 255		Controls *N* = 459	
Characteristic	***N***	**(%)**	***N***	**(%)**
Hospital	85	33.3	151	32.9
Residence				
City of Turin	104	40.0	206	44.1
Turin province	156	60.0	261	55.9
Educational level				
Junior high school	95	36.5	163	35.1
High school	107	41.1	204	43.9
University degree	58	22.3	98	21.1
Missing	0		2	
Cryptorchidism[a]				
No	226	88.6	445	97.0
Yes	29	11.4	14	3.0
Histology				
Seminomas	137	53.7	/	/
Non-seminomasb	117	45.9	/	/
Missing	1			

- [a] Confirmed by a physician.
- [b] Including mixed germ-cell tumours.

Table 2 summarizes the results on exposures related to physical activity. Having done at least one competitive sport at age 13 years, gardening activities at age 13 years and having a lower weight than peers at age 13 years were all inversely associated with the risk of testicular cancer. For gardening there was also some evidence of decreasing risk with increasing frequency (*p*-values: 0.05), while there was no evidence of heterogeneity in the ORs of testicular cancer by sport type.

Table 2. Physical activity during puberty and testicular cancer risk

Variable	No. of cases (%)	No. of controls (%)	OR1a	95% CI	OR2b	95% CI
Sport at age 13 years						
No	152 (61.3)	246 (54.8)	1.00	Ref	1.00	Ref
Yes	96 (38.7)	203 (46.2)	0.74	0.54–1.02	0.72	0.52–1.00
Missing	7	10				
Type of sport						
No sport	152	246	1.00	Ref	1.00	Ref
Ball games	76	159	0.75	0.53–1.06	0.74	0.52–1.05
Weight dependent	8	14	0.89	0.36–2.19	0.86	0.35–2.13
Endurance	25	48	0.81	0.48–1.38	0.77	0.45–1.33
Technical	14	20	1.10	0.53–2.28	1.03	0.49–2.19
Missing	7	13				
Gardening at age 13 years						
No	203 (82.2)	334 (75.2)	1.00	Ref	1.00	Ref
Yes	44 (17.8)	110 (24.8)	0.64	0.43–0.96	0.62	0.42–0.94

Variable	No. of cases (%)	No. of controls (%)	OR1a	95% CI	OR2b	95% CI
Missing	8	15				
Frequency of gardening						
No Gardening	203 (82.9)	334 (77.3)	1.00	Ref	1.00	Ref
<4 times in a month	11 (4.5)	27 (6.3)	0.65	0.31–1.35	0.60	0.28–1.28
4–7 time in a month	27 (11.0)	47 (10.9)	0.92	0.56–1.53	0.92	0.55–1.54
>7 times in a month	4(1.6)	24 (5.6)	0.26	0.26–0.77	0.28	0.10–0.83
Missing	10	27				
p-Value for trend			0.04		0.05	
Weight at age 13 years						
Same as peers	130 (51.4)	208 (45.8)	1.00	Ref	1.00	Ref
Lower than peers	70 (27.7)	164 (36.1)	0.68	0.48–0.97	0.64	0.42–0.97
Higher than peers	53 (20.9)	82 (18.1)	1.01	0.66–1.52	1.01	0.65–1.57
Missing	2	5				

- Abbreviations: CI, confidence interval; Ref, reference.
- [a] OR1, Odds ratio adjusted for age at diagnosis, year of birth, residence (city of Turin, rest of the Turin province), method of identification/contact.
- [b] OR2, Odds ratio adjusted for age at diagnosis, year of birth, residence (city of Turin, rest of the Turin province), method of identification/contact, educational level (junior high school, high school, university degree), cryptorchidism and, for weight at age 13 years, for height at age 13 years.

Results on tobacco and alcohol exposure at age 13 years are reported in Table 3. Confidence intervals were wide for all exposures and there was no evidence of association of active and passive smoking and alcohol consumption with the risk of testicular cancer.

Table 3. Lifestyle during puberty and testicular cancer risk

Variable	No. of cases (%)	No. of controls (%)	OR1a	95% CI	OR2b	95% CI
Parental smoking at home at age 13 years						
No	96 (40.9)	184 (44.9)	1.00	Ref	1.00	Ref
Yes	139 (59.2)	226 (55.1)	1.21	0.87–1.69	1.22	0.87–1.71
Missing	20	49				
Smoking at age 13 years						
No	206 (86.9)	345 (83.3)	1.00	Ref	1.00	Ref
Yes	31 (13.1)	69 (16.7)	0.75	0.47–1.19	0.71	0.44–1.14
Missing	18	45				
Alcohol consumption at age 13 years						
No	207 (81.5)	371 (81.4)	1.00	Ref	1.00	Ref
Yes	47 (18.5)	85 (18.6)	1.04	0.70–1.56	1.03	0.69–1.56
Missing	1	3				
Having been drunk at least once at age 13 years						
No	222 (90.2)	404 (92.5)	1.00	Ref	1.00	Ref
Yes	24(9.8)	33(7.5)	1.33	0.76–2.31	1.28	0.73–2.26

Variable	No. of cases (%)	No. of controls (%)	OR1a	95% CI	OR2b	95% CI
Missing	9	22				

- Abbreviations: CI, confidence interval; Ref, reference.
- [a] OR1, Odds ratio adjusted for age at diagnosis, year of birth, residence (city of Turin, rest of the Turin province), method of identification/contact.
- [b] OR2, Odds ratio adjusted for age at diagnosis, year of birth, residence (city of Turin, rest of the Turin province), method of identification/contact, educational level (junior high school, high school, university degree) and cryptorchidism.

The analysis on agriculture-related occupations revealed consistent results between occasional jobs at age 13 years and having had an occupation up to age 19 years. As shown in Table 4, OR estimates were increased for both variables (OR: 2.40, 95% CI: 1.08–5.29 for occasional jobs during puberty; OR: 2.59, 95% CI: 0.83–8.10 for ever employment in adolescence). There was some evidence of an increased risk of testicular cancer in association with occupational exposure to pesticides, but the OR was only slightly elevated and confidence intervals were wide. Results of the exploratory analysis on all occupations (including those not related to agriculture) are reported in Supplementary Table S1. The median number of job periods held between 13 and 19 years of age for subjects who reported at least 1 job period was 1 and the average number was 1.30 job periods (1.29 for cases and 1.31 for controls). There was no clear evidence of association with the risk of testicular cancer for any occupation, with the possible exception of bricklayers.

Table 4. Agriculture-related exposures and testicular cancer risk

Variable	No. of cases (%)	No. of controls (%)	OR1a	95% CI	OR2a	95% CI
*Agriculture-related occupation*b						
Occasional job at age 13 years						
No	234 (93.2)	427 (95.3)	1.00	Ref	1.00	Ref
Yes	17 (6.8)	21 (4.7)	1.52	0.78–3.00	2.40	1.08–5.29
Missing	7	18				
Ever employed up to age 19 years						
No	241 (97.2)	434 (98.4)	1.00	Ref	1.00	Ref
Yes	7 (2.8)	7 (1.6)	1.97	0.67–5.80	2.59	0.83–8.10
Missing	4	11				
Occupational exposure to pesticides						
Exposure up to age 19 years						
No	249 (98.2)	249 (97.7)	1.00	Ref	1.00	Ref
Yes	6 (2.4)	8 (1.74)	1.32	0.45–3.88	1.78	0.59–5.43
Missing	/	/				
Duration of exposure						
Not Exposed			1.00	Ref	1.00	Ref
Increase in 1 year of exposure		1.06	0.91–1.23	1.11	0.95–1.30	

- Abbreviations: CI, confidence interval; Ref, reference.
- [a] OR1, Odds ratio adjusted for age at diagnosis, year of birth, residence (city of Turin, rest of the Turin province), method of identification/contact; OR2, Odds ratio adjusted for age at diagnosis, year of birth, residence (city of Turin, rest of the Turin province), method of identification/contact, educational level (junior high school, high school, university degree), cryptorchidism, gardening, rural area.
- [b] Agriculture-related occupations: ISCO-88 code 1311 (Manager in agriculture, forestry and fishing); ISCO-88 code 611 (Market gardeners and crop growers), ISCO-88 code 921 (Agricultural, fishery and related labourers).

Table 5 reports the results on grouped causes of hospitalization up to age 18 years. Having had at least one hospitalization for any cause (excluding cryptorchidism) was associated with an increased risk of testicular cancer. Specifically, the risk was increased for the two most frequent causes (adenoidectomy or tonsillectomy and appendectomy) but not for the third most common cause (infections). Two cases and no control had been hospitalized for Type 1 diabetes. Hospitalization for genital malformations (excluding cryptorchidism) was weakly but positively associated with the risk of testicular cancer, with wide confidence intervals. Lastly, when compared to men with no history of cryptorchidism, there was a higher risk of testicular cancer for men who had still cryptorchidism at age 15 years (OR: 5.50, 95% CI 1.72–17.54) than for men whose cryptorchidism resolved before that age (OR: 3.46, 95% CI: 1.71–6.00) (data not shown in tables).

Table 5. Medical conditions and testicular cancer risk

	N° Ca/Coa	**OR1b**	**95% CI**	**OR2b**	**95% CI**
Any hospitalization up to 18 years of age (excl. cryptorchidism)	92/153	1.59	1.13–2.22	1.57	1.11–2.21
Disease/intervention groups					
Genital malformations (excl. cryptorchidism)	5/6	1.31	0.36–4.73	1.25	0.35–4.54
Non genital malformations and genetic syndromes	12/20	1.12	0.54–2.35	1.12	0.53–2.38
Infections	16/37	0.88	0.47–1.63	0.87	0.46–1.63
Trauma or bones fractures	3/7	0.76	0.19–2.96	0.84	0.21–3.28
Asthma or atopic status	6/5	2.08	0.62–6.94	1.75	0.50–6.02
Type I diabetes	2/0	/[3]	/	/	/
Tonsillitis/tonsillectomy or adenoiditis/adenoidectomy	37/39	1.87	1.16–3.03	1.89	1.16–3.09
Appendectomy	21/26	1.51	0.83–2.75	1.68	0.92–3.08
Other diseases	12/24	0.91	0.45–1.87	0.92	0.45–1.90

- Abbreviation: CI, confidence interval.
- [a] Exposed cases/controls.

- [b] OR1, Odds ratio adjusted for age at diagnosis, year of birth, residence (City of Turin, rest of the Turin province), method of identification/contact; OR2, Odds adjusted for age at diagnosis, year of birth, residence (City of Turin, rest of the Turin province), method of identification/contact, educational level (junior high school, high school, University degree) and cryptorchidism.
- [c] Results not show since exposed subjects are <10.

Discussion

The EPSAM study investigated postnatal exposures in relationship with the risk of testicular cancer, with particular focus on exposures occurring in puberty or early adolescence. We found that competitive sports, gardening and low weight at age 13 years were associated with a decreased risk of testicular cancer, while there was an increased risk for working in agricultural-related occupations at age 13 years or adolescence. There was no evidence of association of tobacco and alcohol consumption at age 13 years with the risk of testicular cancer.

For some of the exposures that we analysed there is some evidence from previous studies, while, to our knowledge, other factors evaluated in our study have not been studied before in association with the risk of testicular cancer.

Only few studies explored the association between physical activity in childhood or adolescence and the risk of testicular cancer [13-16]. They all assessed the exposure retrospectively and used slightly different definitions of physical activity. Gallagher *et al*. observed an inverse association and grouped together recreational and sport activities considering the whole life history before the diagnosis of testicular cancer. Cook *et al*. found an inverse association when analysing maternal recall of vigorous physical activity or sport in childhood or adolescence, while they found no evidence of association when they used exposure information reported by the index subjects. A third study reported no association between frequency of strenuous activity and duration of competitive sports in adolescence with the risk of testicular cancer, and only one study found that strenuous recreational activity in the teens was positively associated with the risk of testicular cancer. However, in the latter study, the OR estimates were constant through the different frequency categories, suggesting that there was mainly a decreased risk among those with a frequency of less than once a month.

In our study, results on physical activity are supported by the finding of a decreased risk of testicular cancer associated with gardening, which may be an important component of the overall activity [17] and is typically mentioned as one of the examples of moderate activity in structured questionnaires. To our knowledge, gardening activity has not been analysed before as a separated exposure in association with the risk of testicular cancer. Our results on weight at age 13 years are also consistent with the possibility that more active adolescents have decreased risk of testicular cancer. A meta-analysis of 12 studies did not find evidence of an association between weight and the risk of testicular cancer [18] but our study specifically focused on age 13 years, and is thus not directly comparable to the previous literature.

Physical activity may affect several physiological factors, including hormonal levels, body fat percentage, insulin-like growth factor levels, cytokines levels and oxidative stress. All these factor could affect the risk of cancer development in different organs [19]. However, regarding testicular cancer, if physical activity has a protective effect, its related changes in hormonal levels might be the most likely underlying mechanism [20].

Tobacco and alcohol consumption have been repeatedly analysed in association with the risk of testicular cancer, revealing overall no evidence of an association [21]. Consistently, our study did not find evidence of association for these exposures, when focusing on puberty. However, a recent case–control study on testicular cancer conducted in the USA analysed alcohol consumption at 12–13 years (i.e., during puberty, as in our study) and at 14–17 years of age (i.e., in adolescence) [22]. The association with the risk of testicular cancer was very weak for puberty but there was a 34% increased risk for alcohol consumption in adolescence.

Our results on agriculture-related occupations are more difficult to interpret and put in the context of the current evidence. We focused specifically on occupations during adolescence and considered also occasional jobs at age 13 years, which were not infrequent in our population and would have been missed in a standard questionnaire on occupational history. Systematic reviews and large registry-based studies do not suggest that farming is associated with the risk of testicular cancer [23-25], but most of these studies did not focus specifically on exposures occurring in puberty or adolescence, and, thus, they should be compared to caution to our study: our results could be false positives but, however, some of the previous studies might have missed the relevant window of exposure. Agriculture-related occupations are of particular interest for testicular cancer as it has been suggested that pesticides might play an aetiological role, for example through their endocrine-disrupting activity [26]. In particular, it has been suggested that chemicals with estrogen-like properties (such as organochlorine pesticides) or with anti-androgenic properties (such as p,p′-DDE and phthalates) could interfere with gonadal development and result in an increased risk of testicular cancer. Our study was admittedly underpowered to directly investigate occupational exposure to pesticides, which was relatively rare in our population (1–2% of control men reported that they had been exposed). Results of previous cohort studies on pesticide users are somewhat conflicting, but do not exclude an increased risk of testicular cancer [27-34].

Apart from agriculture-related occupations, the OR of testicular cancer was increased only among bricklayers, with an increased risk for employment before age 19 years, but not for occasional jobs at age 13 years. Bricklayers have a recognized potential for exposure to carcinogens, but are a broad occupational group involving a heterogeneous mix of exposures [35], and we were unable to explore this association further to identify putative agents.

Our results on medical history and the observed increased risk of testicular cancer associated with adenoidectomies or tonsillectomies should be considered with caution, as they might be affected by recall bias. It is however reassuring that the risk was not increased for every group of medical conditions (e.g., the OR was below unity for infections, the third most commonly reported cause of hospitalization among cases and controls) and that the increased risk for congenital malformations different from cryptorchidism (for which recall bias would be expected to be the largest) was weak. To our knowledge, only two previous studies have analysed early tonsillectomy in relation to testicular cancer [36, 37], both finding weak evidence of an increased risk (Whittermore: RR 1.4, 95% CI 0.4–2.7; Swerdlow: OR 1.07, 95% CI 0.75–1.52). Tonsillectomy has been positively associated with different cancer types, such as Hodgkin's disease, breast cancer, leukemia and prostate cancer [38]. As the tonsils are involved in the immune defense it has been suggested that subjects who underwent their removal may have an impaired immunity response, either for the indication for tonsillectomy or as an effect of the lack of tonsils [39, 40].

Finally, even if confidence intervals were largely overlapping, our result of a higher increase in the risk of testicular cancer when cryptorchidism was still unresolved at age 15 years, compared to resolved cryptorchidism at an earlier age, is consistent with previous studies, strengthening the hypothesis that the pubertal period is a specific time window of susceptibility for testicular cancer.

Our study has two main limitations. First, we had a relatively low participation proportion among cases and controls. A response rate of about 50% is common in testicular cancer studies, which target young adults; nevertheless, this may be a source of bias. However, our study focused on exposures that occurred during puberty and these are unlikely to be directly associated with participation. Furthermore alcohol and tobacco consumption, which are well-established determinants of non-response, were not associated with the risk of testicular cancer in our study. This finding is consistent with the literature and indirectly suggests a limited impact of non-response bias on the validity of our results. The second main limitation of our study is that all exposures were self-reported, a feature that is shared with most of the previous studies on postnatal risk factors for testicular cancer. We designed our questionnaire specifically to enhance the possibility of a valid and possibly accurate recall of the exposure. For this reason, for example, we asked only to report competitive sports instead of overall vigorous or moderate activity: this guarantees a higher sensitivity and specificity of the exposure assessment. In addition the focus on a specific time window may have facilitated the recall. For other exposures, for example weight at age 13 years compared to peers, it is likely that there was a non-negligible degree of misclassification, which, however, is likely to be non-differential.

Taken these limitations into account this is, to our knowledge, the first study that has attempted to explore several postnatal exposures acting specifically during puberty or adolescence in association with the risk of testicular cancer. Our main findings suggest that being active during puberty may be inversely associated with the risk of testicular cancer, while working in agriculture-related occupations during puberty or in adolescence is associated with an increased risk. These results need replication, but they underline that postnatal exposures acting in specific time windows may play a role in testicular cancer aetiology, and this would have strong implications for the prevention of a cancer that is believed to originate mainly prenatally.

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