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ARTICLE

Chronic lymphocytic leukemia

Antimicrobial use before chronic lymphocytic leukemia: a retrospective cohort study

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Abstract

Chronic lymphocytic leukemia (CLL) is accompanied by increased risk of potentially fatal infections. While this can mostly be attributed to disease-related immune dysfunction, it is not known if CLL patients are also constitutionally susceptible to infections. We linked nation-wide Danish registers to explore this possibility, approximating infection susceptibility by use of antimicrobials. We assessed the incidence of antimicrobials among CLL patients and matched controls from the general population for up to 22 years before index diagnosis, and among children and grandchildren of CLL patients and their matched controls. Our analyses showed that for CLL patients overall antimicrobial use began to increase gradually six years before leukemia diagnosis. Before this time point, CLL patients had used significantly more macrolides (relative risk = 1.15; 95% confidence interval 1.10–1.20), antimycotics (1.18; 1.08–1.30), and antivirals (1.62; 1.45–1.81) than controls for up to 22 years before diagnosis. The same pattern of increased use was found among CLL patients' children and grandchildren. Our study suggests that CLL diagnosis is preceded by decades of increased susceptibility to infections. The duration of this time window is compatible with causal roles of immune dysfunction and/or certain infections in CLL pathogenesis, possibly mediating the association between constitutional infection susceptibility and CLL risk.

Background

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL) is a monoclonal disorder characterized by the accumulation of mature but functionally incompetent B-lymphocytes.

CLL develops by serial acquisition of somatic mutations accumulating over years or even decades [1]. Consistent with its progressive development, CLL is preceded by a pre-leukemic state, monoclonal B-lymphocytosis (MBL) [2], and both MBL and CLL are accompanied by an increased risk of infections [3]. This is believed to explain why CLL may be preceded by excessive infections, in particular pneumonias, for up to 10 years before diagnosis [4–7].

More intriguingly, the increased occurrence of infections before CLL diagnosis could also be informative regarding CLL pathogenesis. Specifically, infections might contribute to CLL development through antigen stimulation, inducing somatic mutations, or epigenetic modifications in line with the antigenic drive in lymphoid malignancies.

Genome-wide association studies (GWAS) have identified multiple loci associated with CLL risk, which also associate with MBL [8]. Several of the gene polymorphisms implicated in CLL risk by the GWAS suggest a key role for aberrant immune responses in CLL pathogenesis [8]. It is not known if these genetic aberrations manifest in other ways than MBL/CLL risk, e.g., whether they contribute to the increased infection risk before (and after or independent of) CLL.

To explore this possibility, we assessed CLL patients' use of antimicrobials for up to 22 years before CLL diagnosis as well as the use among CLL patients' children and grandchildren in a nation-wide register-based investigation.

Supplementary information The online version of this article (https://doi.org/10.1038/s41375-020-0980-0) contains supplementary material, which is available to authorized users.

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Table 1 Descriptive characteristics of the study cohort of all Danish CLL patients ($n = 8161$) diagnosed 1996–2017 and their controls ($n = 47,172$) and children ($n = 14,052$) and grandchildren ($N = 22,815$) with their respective controls ($n = 70,454$ and $n = 113,636$). The number of prescriptions for each of investigated antimicrobials.

Case/control	Relation	Number	Mean age	SD	IQR-L	IQR-U	Person years	Any	J01A	J01C	J01E	J01F	J01M	J01X	J02A	J05A	P01A	P02C
Controls	CLL	47172	70.4	11.1	63.1	78.5	539808	220443	9160	167941	32225	46692	11185	10239	11389	7744	6912	1194
Cases	CLL	8161	71.0	11.2	63.6	79.2	96875	43026	1899	33107	5970	10165	2263	1747	2168	2110	1341	239
Controls	Child	70445	36.8	10.0	31.0	43.0	7802241	261866	14275	221426	24565	64831	11081	5299	30394	17108	16161	7579
Cases	Child	14052	36.7	10.1	30.8	42.9	1597319	54032	3222	44944	5263	13672	2687	1190	6745	3875	3338	1587
Controls	Grandchild	113636	10.0	9.0	21.3	15.5	1195951	363446	23368	314944	26431	76417	7621	3425	30769	20033	14117	22637
Cases	Grandchild	22815	10.1	9.0	22.4	15.5	250620	76321	5214	65533	5772	16679	1729	850	7182	4367	2951	5069

Methods

We performed a cohort study among all CLL patients diagnosed and their relatives in Denmark between 1 July 1996 and 31 Dec 2017 (last date for which data were available).

Cohort assembly and statistical methods are described in full in appendix. Briefly, from the Danish Cancer Register (DCR) and Danish National Patient Register (DPR), we identified all patients registered with CLL (*ICD10: C911*).

We followed all CLL cases and their controls for antimicrobials use from July 1996, date of birth or date of immigration whichever came last until index date (Supplementary Appendix Figs. 1 and 2).

We followed all relatives to a CLL patient and their matched control, themselves being free from CLL from 1 July 1996, date of birth, date of immigration or date of index-CLL diagnosis whichever came last until death, emigration, date of CLL (relative's own CLL diagnosis) or 31 Dec 2017 whichever came first.

For all individuals, we obtained information on systemic antimicrobial prescriptions (ATC codes J01A, J01C, J01E, J01F, J01M, J02A, J05A, P01A, P02C) from the Danish National Prescription Register. From DPR we retrieved data on all hospital diagnoses within the study period.

We used Poisson regression models with log expected counts as offset to produce standardized incidence ratios of antimicrobial use among (future) CLL cases in one-month intervals [9]. Throughout we denote these standardized incidence ratios as relative risks.

Results

Between 1 July 1996 and 31 Dec 2017, we identified 8161 individuals diagnosed with CLL and matched them to 47172 CLL-free individuals. Mean age at index date was 71.0 years (interquartile range (IQR) 63.6–79.2 years); mean follow-up was 11.8 years (standard deviation (SD) 6.5 years); most patients (58.6%) were males (Table 1). Approximately 25% of both cases and controls used antimicrobials in a given year during the study period. More women (79% for controls and 83% for cases) than men (74% for controls and 82% for cases) in the cohort had used antimicrobial during follow up (Supplementary Appendix Table 1). Comorbidities were similarly distributed among cases and controls (Supplementary Appendix Table 2).

The most commonly redeemed antimicrobial was β-lactam antimicrobials (J01C), of which phenoxymethylpenicillin (J01C02) constituted 59%. Macrolides (J01F) was the second most common antimicrobial type (Supplementary Appendix Fig. 3).

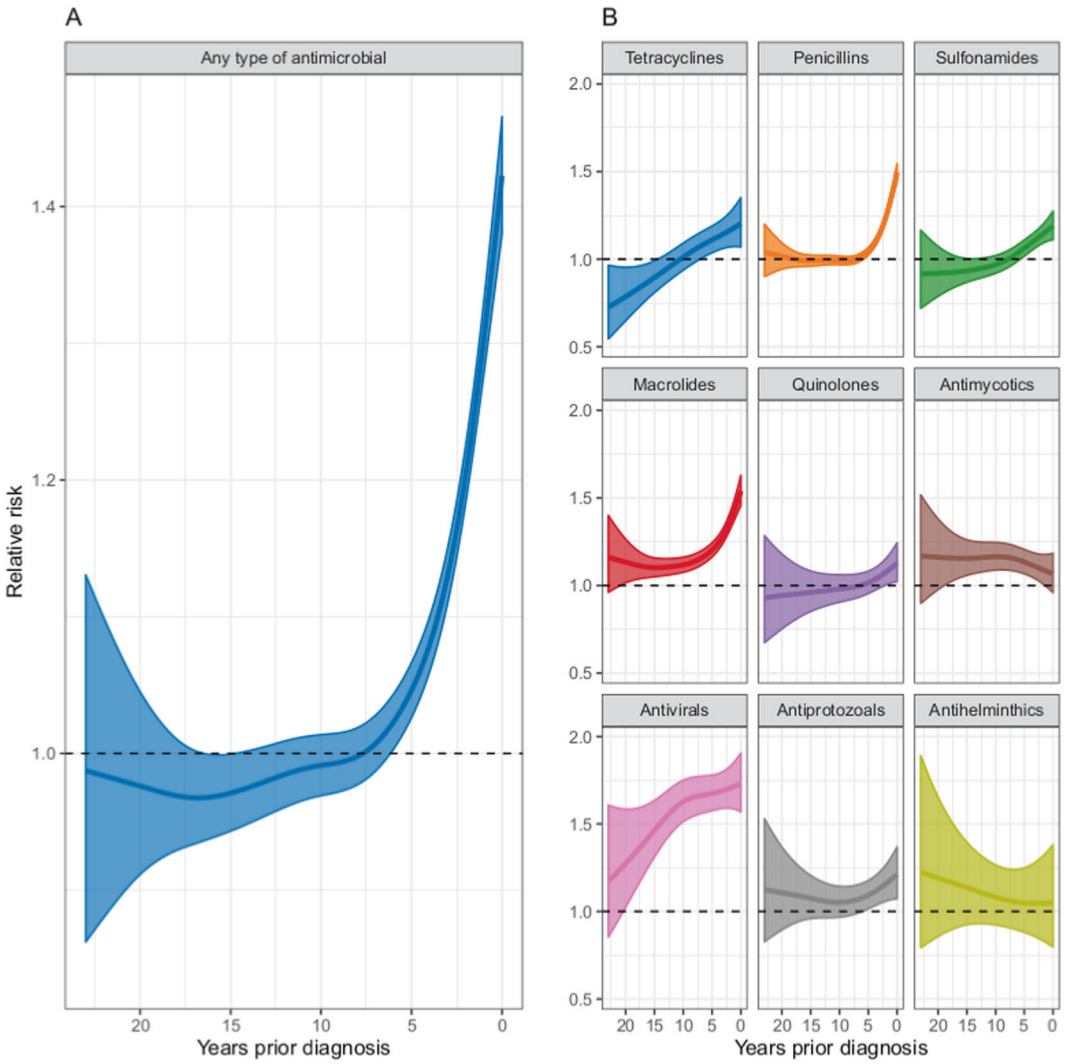

Fig. 1 Antimicrobial use before chronic lymphocytic leukemia. **a** Use of any type antimicrobials in CLL patients relative to controls. Relative risk with 95% confidence intervals for use of specific antimicrobials patients relative to controls. **b** Use of specific antimicrobials relative to controls. Inspection of graphs of antimicrobial use for cases and controls revealed that our results were not driven by only few individuals (data not shown).

At index date, CLL patients had a higher intensity of antimicrobial use compared with control individuals (0.72 vs. 0.53 antimicrobials per person year) (Supplementary Appendix Fig. 4). The difference in overall antimicrobial exposure between CLL patients and controls correlated inversely with time to diagnosis index date and was statistically significant in the 6 years immediately before CLL diagnosis (Fig. 1a). Before this time-point, overall use of antimicrobials was identical among cases and controls. The same temporal pattern was observed for most specific types of antimicrobials investigated, i.e., they had been used statistically significantly more often among patients than controls in the 6 years immediately preceding the diagnosis (Fig. 1b). However, in addition to this late phenomenon macrolides (RR = 1.15 [95% confidence interval 1.10–1.20]), antivirals (RR = 1.62 [1.45–1.81]), and antimycotics (RR = 1.18 [1.08–1.30]) had been used more frequently by CLL patients throughout all time windows before CLL diagnosis (Fig. 1; Supplementary Appendix Fig. 5).

During the study, 33 children of CLL patients developed CLL and were no longer followed as exposed, no grandchildren developed CLL during follow-up. For further characteristics, see Table 1.

Compared with controls children and grandchildren of CLL patients used significantly more macrolides (RR_{child} = 1.03 [1.02–1.05], $RR_{grandhild}$ = 1.02 [1.01–1.04]), tetracyclines (RR_{child} = 1.09 [1.05–1.13], $RR_{grandhild}$ = 1.04 [1.01–1.07]), antivirals(RR_{child} = 1.08 [1.05–1.11], $RR_{grandhild}$ = 1.04 [1.01–1.07]), antimycotics (RR_{child} = 1.07 [1.05–1.10], $RR_{grandhild}$ = 1.09 [1.06–1.11]), antihelminthics (RR_{child} = 1.05 [1.00–1.10], $RR_{grandhild}$ = 1.06 [1.03–1.09]) (Supplementary Appendix Fig. 6).

Discussion

Taking advantage of nation-wide registers, we compared use of antimicrobials among CLL patients and controls for up to 22 years before leukemia diagnosis and found evidence of two separate yet consecutive trajectories.

Firstly, our analyses showed that overall antimicrobial use started to gradually increase among future CLL patients from 6 years before their leukemia diagnosis.

Secondly, we found that preceding the increasing use heralding impending CLL diagnosis the patients had already been using certain antimicrobials more often than the population controls for more than 10 5 years. Indeed, the use of macrolides, antivirals and antimycotics were significantly increased for up to 22 years before the CLL diagnosis.

Our observations support and expand the current literature on the association between infections and CLL risk. Specifically, register-based investigations have previously reported that infection risk increases as CLL diagnosis approaches [4–7]. This temporal development is consistent with the notion that the increasing infection susceptibility leading up to CLL diagnosis are manifestations of immune dysfunction resulting from MBL and/or undiagnosed CLL. MBL is a precursor of CLL and is associated with an increased risk of infections [3]. In retrospective investigations MBL has been demonstrated up to 77 months in advance of CLL diagnosis and serum immunoglobulin abnormalities in up to 112 months before CLL diagnosis [2, 10]. Moreover, the temporal distribution of infection risk closely mirrors the progressive development of lymphocytosis before CLL diagnosis [11].

The rise in antimicrobial use in the last years before CLL diagnosis was preceded by a much longer period of increased use of certain antimicrobials, specifically macrolides, antivirals and antimycotics, which is less easily attributable to the same mechanisms. Though less detailed with respect to timing than the present study, one study partially overlapping with the present reported similar elevated risks of antibiotics (up to 10 years before CLL diagnosis) [7], and other studies have reported increased risk of cellulitis (>5 years), chronic rhinosinusitis (>10 years), and herpes zoster (>10 years) [4–7]. Our findings concerning use of macrolides and antivirals for extended periods before CLL diagnosis can easily be extrapolated to these observations, since these are recommended drugs for treatment of chronic rhinosinusitis and herpes zoster, respectively.

This suggests that our observations are not merely chance findings and calls for speculations regarding the underlying mechanisms. One possibility is that the increased infection susceptibility 20 years prior to diagnosis are manifestations of immune dysfunction resulting from MBL and/or undiagnosed CLL. An alternative explanation is that the susceptibility is a consequence of constitutional predisposition to CLL. Accordingly, GWAS have identified multiple loci annotating genes that are central for B-cell development, suggesting that immune dysfunction is critical to CLL development [8].

Our analyses of antimicrobial use among children and grandchildren of CLL patients further support this hypothesis. Specifically, we observed elevated use of macrolides, antivirals and antimycotics among these relatives, estimates of relative risk being higher among children than among grandchildren consistent with an inheritable trait. This explanation would not be without precedence in hematological malignancies, as other hematological cancers with a more abrupt onset like Hodgkin lymphoma display inherited immune dysregulation [12].

Our investigation has several strengths. We combined nation-wide administrative and research registers to establish unique population-based sets of CLL patients and controls [7]. These two groups were followed for up to 22 years before index diagnosis with inpatient-register information allowing adjustments for comorbidities.

Several limitations must also be considered. CLL is often asymptomatic and may be diagnosed by chance in connection with health care visits for other conditions. This may lead to an ascertainment bias as the risk of being diagnosed with CLL may be higher among heavy users of antibiotics, for example in connection with routine follow up visits. However, unless explained by other conditions requiring antimicrobials treatment running in families predisposed to CLL such ascertainment bias would not explain our findings among CLL patient relatives.

CLL and MBL share genetic risk factors, as also evidenced by GWAS and the high prevalence of MBL among CLL relatives [13]. The precise mechanism by which MBL develops is not known, but its prevalence increases with age from being virtually absent before the age of 30 years to 5% or more among the elderly [14]. This suggests that some form of cumulative exposure, possibly infections, drives the development. Consistent with this, a cross-sectional study observed lower prevalence of MBL among persons vaccinated for influenza or pneumococci [15]. Thus, further studies unravelling the interplay between genetic risk and antigenic drive decades prior to CLL diagnosis are warranted.

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Data sharing Danish registry data can be obtained through application to the relevant data agencies. Analytical methods and source code will be made available on request to corresponding author.

Compliance with ethical standards

Conflict of interest CUN received funding from the Danish Cancer Society and the Novo Nordisk Foundation (NNF16OC0019302), and received grants/consultancy fees from AbbVie, Janssen, Gilead, AstraZeneca, Acerta, CSL Behring Roche and Novartis outside of this study. MAA, HH, and KR report no conflict of interest.

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