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Monoclonal B-cell lymphocytosis in a hospital-based UK population and a rural Ugandan population: a cross-sectional study

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Summary

Background Reported incidence of B-cell malignancies shows substantial geographical variation, being more common in the Americas and Europe than in Africa. This variation might reflect differences in diagnostic capability, inherited susceptibility, and infectious exposures. Monoclonal B-cell lymphocytosis (MBL) is a precursor lesion that can be screened for in apparently healthy people, allowing comparison of prevalence across different populations independently of health-care provision. We aimed to compare the prevalence and phenotypic characteristics of MBL in age-and-sex-matched populations from rural Uganda and the UK.

Methods In this cross-sectional study, we recruited volunteers aged at least 45 years who were seronegative for HIV-1 from the established Ugandan General Population Cohort and obtained their whole-blood samples. We also obtained blood samples from anonymised waste material of age-and-sex-matched individuals (aged >45 years, with a normal blood count and no history of cancer) in the UK. We used flow cytometry to determine the presence of MBL, defined according to standard diagnostic criteria, in the samples and compared differences in the proportion of cases with chronic lymphocytic leukaemia (CLL)-phenotype MBL and CD5-negative MBL, as well as differences in absolute monoclonal B-cell count between the two cohorts.

Findings Between Jan 15 and Dec 18, 2012, we obtained samples from 302 Ugandan volunteers and 302 UK individuals who were matched by age and sex to the Ugandan population. Overall MBL prevalence was higher in the Ugandan participants (42 [14%] individuals) than in the UK cohort (25 [8%]; p=0·038). CLL-phenotype MBL was detected in three (1%) Ugandan participants and 21 (7%) UK participants (p=0·00021); all three Ugandan participants had absolute monoclonal B-cell count below one cell per µL, whereas the 21 UK participants had a median absolute number of circulating neoplastic cells of 4·6 (IQR 2–12) cells per µL. The prevalence of CD5-negative MBL was higher in the Ugandan cohort (41 [14%], of whom two [5%] also had CLL-phenotype MBL) than in the UK cohort (six [2%], of whom two [33%] also had CLL-phenotype MBL; p<0·0001), but the median absolute B-cell count was similar (227 [IQR 152–345] cells per µL in the Ugandan cohort *vs* 135 [105–177] cells per µL in the UK cohort; p=0·13).

Interpretation MBL is common in both Uganda and the UK, but the substantial phenotypic differences might reflect fundamental differences in the pathogenesis of B-cell lymphoproliferative disorders.

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Introduction

B-lymphoproliferative disorders include a wide range of both non-malignant and malignant conditions. The malignant diseases represent the most frequent adult haematological cancers, accounting for more than 50% of leukaemia and more than 90% of non-Hodgkin lymphoma diagnoses.[1] However, major geographical variation exists in the reported incidence of B-lymphoproliferative disorders. According to GLOBOCAN 2012,[2] age-standardised incidence per 100 000 person-years for leukaemia and non-Hodgkin lymphoma in the WHO African region is 6·4, compared with 18·2 in the WHO Americas region and 21·3 in the WHO European region. Chronic lymphocytic leukaemia (CLL) is the most common adult leukaemia in Europe and the USA but incidence seems to be low in Africa, with some data suggesting that other B-cell malignancies, such as lymphoplasmacytic lymphoma and marginal zone lymphomas, might be more frequent than CLL.[3] Several factors can affect the reported differences in incidence, including variable quality of access to cancer care, differences in age distribution, and failure to diagnose B-lymphoproliferative disorders, especially in the elderly.[2] Monoclonal B-cell lymphocytosis (MBL) is a precursor lesion of B-lymphoproliferative disorders and provides a useful model for the study of B-cell lymphomagenesis, since it can be screened for in otherwise healthy people,[4–6] thereby allowing a comparison of prevalence across geographical regions in a way that is independent of health-care provision. MBL with a CLL phenotype is

Research in context

Evidence before this study

Monoclonal B-cell lymphocytosis (MBL) is a precursor to B-cell haematological malignancy that can be used as a model for the study of B-cell lymphomagenesis. MBL with a chronic lymphocytic leukaemia phenotype is common in Europe and the USA and, compared with other MBL subtypes, has been relatively well characterised in terms of phenotype, genotype, and B-cell receptor immunoglobulin gene repertoire. Furthermore, the prevalence, rate of disease progression, and inherited susceptibility single-nucleotide polymorphisms patterns are well established in the USA and Europe. We found no reports of MBL prevalence in Africa on PubMed up to Dec 31, 2015 (search term "monoclonal B-cell lymphocytosis Africa").

Added value of this study

To our knowledge, this is the first study to provide data for the prevalence and phenotype of MBL in an African country. Overall MBL prevalence was slightly higher in rural Uganda than in the UK but chronic lymphocytic leukaemia-phenotype MBL was infrequent whereas CD5-negative MBL was much more common in rural Uganda and showed substantial phenotypic differences compared with the UK population.

Implications of all the available evidence

Although MBL is common in both rural Uganda and the UK, the variation in phenotype shows that differences in environmental exposure, inherited susceptibility, or both factors affect the type of B-cell neoplasms that can develop.

common in Europe and the USA and, compared with other MBL subtypes, has been relatively well characterised in terms of phenotype, genotype, and B-cell receptor immunoglobulin gene repertoire.[7–13] Furthermore, the incidence, rate of disease progression, and inherited susceptibility single-nucleotide polymorphism (SNP) patterns are well established in US and European populations.[14–17] MBL of non-CLL phenotype is also increasingly recognised, and preliminary data suggest that this subtype is less common than CLL-phenotype MBL[6,18,19] but might be a precursor of entities within the spectrum of marginal zone lymphomas, typically of splenic type.[20,21]

Therefore, the study of MBL in Uganda might provide insights into the pathogenesis of B-lymphoproliferative disorders in sub-Saharan Africa, and comparison with data from the UK could deepen these insights. In this study, we aimed to assess the prevalence of MBL in a rural population in Uganda and compare these findings with those from a study of MBL in a UK population.

Methods

Study design and data collection

In this cross-sectional study, we analysed samples from participants in rural Uganda and a UK hospital-based population (appendix pp 1–2). We recruited volunteers aged at least 45 years who were seronegative for HIV-1 from the Ugandan General Population Cohort, a community-based open dynamic cohort with more than 22 000 people at present. Whole-blood samples were obtained from the participants, stored at 4°C overnight, and analysed within 24 h. We used a previously reported method[16] to obtain whole-blood samples from anonymised waste material of UK hospital outpatients and primary care patients with a normal blood count and no history of cancer. These patients were selected to match the age and sex distribution of the Ugandan participants.

See Online for appendix

Ethics approval for sample collection in Uganda was provided by the Uganda Virus Research Institute Scientific Ethics Committee and the Uganda Council for Science and Technology, and for sample collection in the UK by the Leeds Teaching Hospitals National Health Service Trust Ethics Review Board. In Uganda, study personnel read the consent form to all participants, who signed or thumb-printed (for those who were illiterate) the forms.

Procedures

MBL is a condition in which the B-cell surface immunoglobulin light chain κ-to-λ ratio is heavily skewed in total B-cells or in a B-cell subset. In previous studies the detection of MBL representing a subset of B cells (ie, with predominantly polyclonal B cells) has been limited to CLL-phenotype cells whereas CD5-negative MBL has represented a skewed κ-to-λ ratio in total B cells.[5,6,18,19] However, in some individuals, immunoglobulin light-chain skewing could be detected within B-cell subsets defined by CXCR5 (CD185) and LAIR1 (CD305) expression. In European populations, these markers are strongly expressed in most normal B cells but show aberrant expression in most B-lymphoproliferative disorders.[22] LAIR1 (CD305) expression varies according to the type of B-lymphoproliferative disorder: germinal-centre B-cell malignancies typically have no LAIR1 expression; Waldenström's macroglobulinaemia usually have either no LAIR1 expression or intermediate levels, often with both a LAIR1-negative and a LAIR1-positive component; and hairy cell leukaemia and splenic lymphomas usually have stronger than normal expression.[22]

We used flow cytometry to determine the presence of MBL, defined according to the standard diagnostic criteria[23]—κ-to-λ ratio greater than 3:1 or less than 0·3:1, or more than 25% of B cells without surface immunoglobulin or expressing low levels of surface immunoglobulin either in the total B-cell population (CD19 positive, CD20 positive), or in one or more pre-specified B-cell subsets: CD5 positive, CD20 weak,

surface immunoglobulin weak (CLL-phenotye); CD10 positive; CD305 positive and CD185 negative; CD305 positive and CD185 positive; CD305 negative and CD185 negative; or CD305 negative and CD185 positive. The threshold for positivity was validated against internal cellular controls in each case.

We analysed the immunoglobulin gene repertoire of 13 Ugandan individuals selected to represent each of the MBL categories (three with CLL-type MBL, three with CD5-negative MBL, three with a monoclonal B-cell subset) and individuals with polyclonal B-cells (four individuals). *IGHV–IGHD–IGHJ* gene rearrangements were amplified from genomic DNA (200 ng, extracted from at least 2×10^6 peripheral blood mononuclear cells) and sequenced according to the standard Roche 454 GS-Junior sequencing protocol. Productive, in-frame sequences were analysed using IMGT HighV-Quest software, version 1.3.0.[24] Genotype data were generated with the Illumina HumanOmni2.5 BeadChip array at the Sanger Institute (Cambridge, UK) for 5000 participants of the General Population Cohort.[25] The data were assessed for six SNPs reported to be associated with both CLL and MBL, and compared with published UK risk-allele frequencies.[14] Comparisons with African American people were made using data from the 1000 Genomes project.

Statistical analysis

No formal power calculations were done because of insufficient data from Africa, and sample size was determined pragmatically. We compared differences in the proportion of cases with CLL-phenotype MBL and CD5-negative MBL and in absolute monoclonal B-cell count between the Ugandan and UK samples. We assessed significance using Fisher's exact test (two-tailed) or the Wilcoxon-Mann-Whitney *U*-test (two-tailed) as appropriate, using IBM SPSS version 24.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Jan 15 and Dec 18, 2012, we obtained samples from 302 Ugandan volunteers and 302 age-and-sex-matched UK individuals. 121 (40%) participants from Uganda were women aged 40–60 years, 47 (16%) were men aged 40–60 years, 90 (30%) were women older than 60 years, and 44 (15%) were men older than 60 years. The UK population was matched exactly by sex and age.

MBL was detected in 42 (14%) of 302 Ugandan participants and 25 (8%) of 302 UK participants (p=0·038). Monoclonal CLL-phenotype B cells were detected in three (1%) Ugandan participants and in 21 (7%) UK participants (p=0·00021; figure 1). By contrast, CD5-negative MBL was more prevalent in the Ugandan participants (41 [14%] individuals, of whom two [5%] also had CLL-phenotype MBL detectable) than in the UK participants (six [2%], of whom two [33%] also had CLL-phenotype MBL detectable; p<0·0001; figure 1). A monoclonal B-cell subset was detectable in 93 (31%) Ugandan participants and 21 (7%) UK participants (p<0·0001; figure 1). CD10-positive monoclonal B-cell populations with a germinal centre phenotype were not detected in either population.

In addition to differences in the phenotype of monoclonal B cells between the two cohorts, the absolute counts of monoclonal B cells also differed significantly (figure 2). All three Ugandan participants with CLL-phenotype MBL had absolute monoclonal B-cell count below one cell per µL and close to the detection limit of the assay. By contrast, in the 21 UK participants with CLL-phenotype MBL, the median absolute number of circulating neoplastic cells was 5 cells per µL (IQR 2–12, range 1–1773). Seven (2%) UK participants had more than ten CLL-phenotype cells per µL, compared with none in the Ugandan participants (p=0·015). Although the prevalence of CD5-negative MBL was higher in the Ugandan participants than in the UK participants, the median absolute B-cell count was similar (227 [IQR 152–345, range 56–947] cells

For the **1000 Genomes project** see http://browser.1000genomes.org/index.html

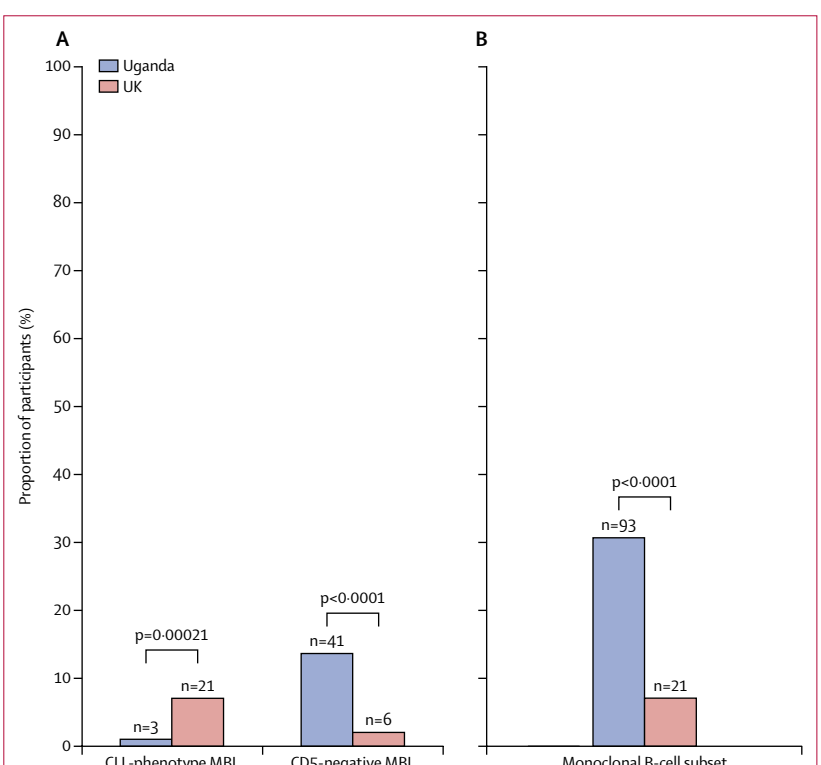

Figure 1: **Prevalence of MBL in the Ugandan and UK cohorts, by B-cell phenotype**
(A) Proportion of participants with detectable CLL-type MBL and CD5-negative MBL according to the 2005 diagnostic criteria.[22] (B) Proportion of participants with a monoclonal B-cell subset. CLL=chronic lymphocytic leukaemia. MBL=monoclonal B-cell lymphocytosis.

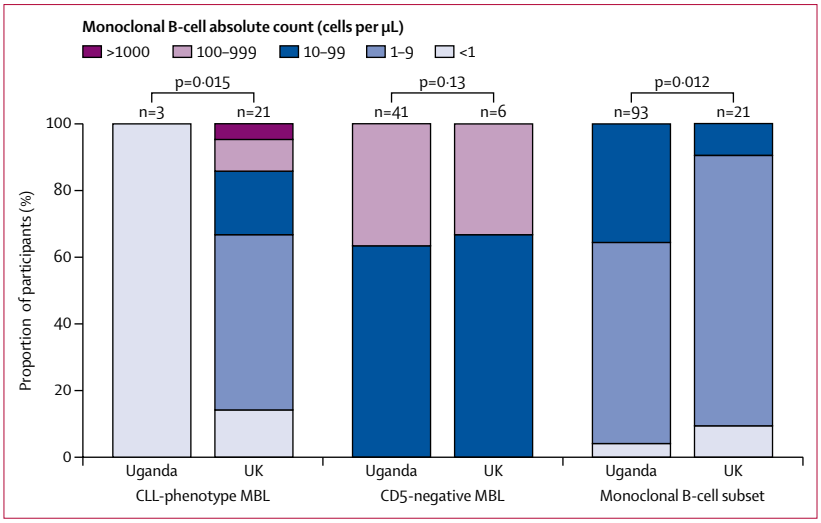

Figure 2: **Absolute monoclonal B-cell count, by phenotype**
CLL=chronic lymphocytic leukaemia. MBL=monoclonal B-cell lymphocytosis.

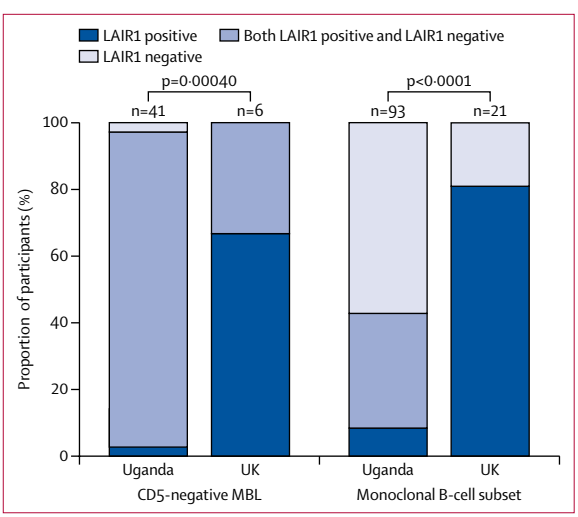

Figure 3: **LAIR1 expression pattern in monoclonal CD5-negative B cells**
MBL=monoclonal B-cell lymphocytosis.

per µL in the Ugandan cohort *vs* 135 [105–177, 69–503] cells per µL in the UK cohort; p=0·13). However, the median absolute count of CD5-negative monoclonal B-cell subsets was significantly higher in the Ugandan cohort (seven [3–15, 1–53] cells per µL) than in the UK cohort (two [2–3, 1–17] cells per µL; p=0·012).

For the **database of CLL sequences** see http://tools.bat.infspire.org/arrest/assignsubsets

Differences were also apparent in LAIR1 expression by CD5-negative MBL and monoclonal B-cell subsets in the two cohorts. Monoclonal B-cell expansions in the UK participants typically showed moderate to strong LAIR1 expression, whereas those in Uganda had weak or bimodal expression similar to the IgM-secreting lymphoplasmacytic disorder (figure 3). Of 41 CD5-negative MBL cases in the Ugandan cohort, 40 (98%) had LAIR1-negative cells or a combination of both LAIR1-negative and LAIR1-positive cells, compared with two (33%) of six UK cases (p=0·0040). Monoclonal B-cell subsets comprised only LAIR1-negative cells or a combination of both LAIR1-negative and LAIR1-positive cells in 85 (91%) of 93 Ugandan cases, compared with four (19%) of 21 UK cases (p<0·0001; figure 3).

The high prevalence of CD5-negative MBL was unexpected; although not initially planned, we extended immunophenotyping on 18 samples from rural Uganda using a panel of 46 markers with a common backbone of CD19, CD20, CD27, CXCR5, and LAIR1 (appendix p 5). The samples were from five individuals with CD5-negative MBL, eight individuals with a monoclonal B-cell subset, and five individuals with polyclonal B-cell subsets. Although the analysis was not powered to show significant differences, some markers—including CD38, CD73, CD200, and CD307d (FcRL4)—had at least a five times difference in expression by CD5-negative MBL B cells compared with polyclonal B cells and at least a two times difference in expression by CD5-negative MBL cells compared with B cells from cases with a monoclonal B-cell subset (appendix p 9). This finding suggests that neoplastic expansions might be phenotypically discriminated in a similar manner to the detection of CLL-phenotype MBL populations.

Immunoglobulin gene repertoire analysis was done in 13 Ugandan individuals, with a median of 224 (range 39–2674, IQR 127-428) productive, in-frame *IGHV–IGHD–IGHJ* gene rearrangements sequenced per individual. Four of these individuals had no detectable MBL clones by flow cytometry and, concordantly, next-generation sequencing did not detect any significant clonal expansion. Three of these individuals were found to carry CD5-negative MBL by flow cytometry, and we detected a clonal population in each one using next-generation sequencing. Three individuals had light-chain restriction detectable by flow cytometry in a subset of B cells, and clonal expansion was identified in one of these individuals (appendix p 6).

In the three individuals with a suspected CLL-phenotype population present at the detection limit of the flow cytometry assay, next-generation sequencing showed no evidence of significant clonal expansion, with the most expanded clonotypes representing a 1·3%, 3·1%, and 3·9% of the B-cell receptor immunoglobulin repertoire, respectively in the three individuals. In a post-hoc analysis, sequences were cross-compared with a database of 19 464 CLL sequences including 668 stereotyped CLL subsets. In one of the three individuals with a suspected CLL-phenotype population, a single, non-expanded clonotype was found (IGHV1-2/IGHD3-3/IGHJ6, CysAlaLysGlyAlaGlnTyrTyrAspPheTrpSerGlyTyrLeu ProTyrTyrTyrGlyMetAspValTrp) clustering to a minor CLL subset. In another individual with a monoclonal B-cell subset, an expanded clonotype consisting of four identical sequences was found (IGHV3-30/IGHD2-21/IGHJ4, CysValLysAspAspGlnTrpGlyProAspTyrTrp) clustering to another minor CLL subset. The third individual had no clonotype clustering to a stereotyped CLL subset.

Genotype data generated for more than 5000 participants of the Ugandan General Population Cohort are shown in the appendix (p 7).[25] The prevalence of six SNPs that have been shown to be significantly associated both with CLL and with CLL-phenotype MBL were compared with published data from the UK[14] on prevalence in the general population and in African Americans. For all of the SNPs associated with CLL-phenotype MBL, prevalence is substantially lower in rural Uganda than in the UK and in African Americans (appendix p 7).

Discussion

In this cross-sectional study, we showed that the prevalence of MBL is marginally higher in rural Uganda than in the UK and that the phenotypes identified are different. By contrast, the incidence of B-lymphoproliferative disorders is reported to be significantly lower in Africa than in Europe and the USA. However, detailed epidemiological studies are compromised by the fact that most patients with indolent B-lymphoproliferative disorders such as CLL and marginal zone lymphomas are asymptomatic and might be less likely to be diagnosed in African countries than in European or North American countries.[2] Therefore, more meaningful data could be obtained with population screening for the precursor disorders. MBL with a CLL phenotype is well characterised, and results from a follow-up study[16] have established that annually roughly 1% of individuals with CLL-phenotype MBL will develop symptomatic CLL that requires treatment. CLL-phenotype B cells are detectable at very low cell counts (often termed low-count MBL, typically $<0{\cdot}01\times10^9$ cells per L) in 3–20% of adults in Europe and the USA, depending on assay sensitivity and age. Individuals with low-count MBL have a similar inherited susceptibility risk allele profile to those with CLL but no evidence of progression to clinical CLL.[12–14,16] CD5-negative MBL is less well characterised, mainly because of a lack of a lack of markers that differentiate neoplastic CD5-negative monoclonal B-cells from reactive monotypic expansions, but the available data suggest that it might be a precursor to marginal zone lymphoma, at least in some cases.[20] However, the use of antigens such as LAIR1 and CXCR5 has improved the diagnosis of CD5-negative B-lymphoproliferative disorders,[22] potentially facilitating the detection of precursor lesions.

Therefore, we used flow cytometry to assess, in detail, the prevalence of MBL in a rural Ugandan population who were HIV negative. We also compared this prevalence with that in an age-and-sex-matched UK control population, since such a comparison might provide insights into the relative incidences of both CLL and marginal zone lymphomas, independent of differences in health-care provision. Monoclonal B-cell populations were found with a similar frequency in adults from rural Uganda and the UK, but the type of monoclonal B-cell expansions seemed distinct. Subpopulations of CLL-phenotype monoclonal B-cells were detected frequently in the UK cohort but very rarely in Ugandan participants. By contrast, a much higher proportion of Ugandan participants had detectable CD5-negative monoclonal B cells in the peripheral blood than adults from the UK.

In a preliminary immunophenotypic analysis, we identified some differences in expression of markers, including CD38, CD73, CD200, and CD307d. In individuals with a predominantly monoclonal B-cell population, the expression pattern suggested neoplastic expansion; by contrast, in individuals with polyclonal B cells or light-chain restriction in a subset of B-cells, the pattern was suggestive of a reactive expansion. The data confirmed that phenotypically identified CD5-negative MBL is consistently associated with clonal B-cell receptor immunoglobulin expansion. We could not confirm the presence of CLL-phenotype monoclonal B cells at the molecular level. Moreover, the presence of non-CLL B-cell subpopulations with light-chain restriction does not necessarily correspond to monoclonal expansions and might reflect a reactive expansion. Further work to identify markers that discriminate between reactive and neoplastic expansions would greatly benefit the diagnostic process for post-germinal centre B-cell disorder.

Further insights have been provided by immunoglobulin gene repertoire analysis and SNP data. Although the numbers of samples included in this study were small, our results suggest that CLL-specific *IGHV–IGHD–IGHJ* gene rearrangements might be detected in the rural Ugandan population without being associated with the detection of a CLL-phenotype MBL. This finding is notable because CLL-derived B-cell receptor immunoglobulin molecules have a central role in CLL pathogenesis by transducing antigen-driven[26] or antigen-independent cell-autonomous signals, or both.[27] Therefore, the presence of CLL-specific rearrangements without clonal expansion indicates that additional or alternative mechanisms are also necessary for the development and expansion of CLL-phenotype MBL. We also confirmed clonality in most CD5-negative MBL cases, although all cases had multiple expanded clonotypes, indicating that reactive expansions might also be common in rural Ugandans. Inherited susceptibility is an important factor in CLL: 30 common genetic variants have been identified so far and six have been confirmed in MBL.[14,28] SNP analysis in the Ugandan cohort confirmed HapMap data showing that CLL susceptibility loci are very rare in this population. Although the absence of CLL susceptibility loci does not preclude development of CLL,[29] some of the SNP are putatively causal for CLL.[30] This finding, in combination with the MBL data, provides strong support for the hypothesis that inherited susceptibility affects the development of specific types of B-lymphoproliferative disorder.

In the UK, the prevalence of MBL subtypes closely reflects the incidence of clinical disease. CLL accounts

for a much higher proportion of B-lymphoproliferative disorders with detectable circulating disease than do other CD5-negative B-cell disorders.[1] In this study, the prevalence of CD5-negative MBL in the UK cohort was similar to that reported in previous studies;[5,6,18,19] however, the inclusion of LAIR1 in the screening process permitted detection of a larger proportion of cases with a monoclonal B-cell subset than has previously been reported. LAIR1 is negative or weakly expressed in most B-cell disorders, moderately expressed on most polyclonal B cells, and strongly expressed in hairy cell leukaemia.[22] Therefore, the use of LAIR1 expression greatly improves the sensitivity for detection of non-CLL-type monoclonal B-cell expansions, although whether these expansions represent a neoplastic precursor lesion or a reactive expansion remains to be determined. The differences seen between the Ugandan cohort and UK cohort in regards to LAIR1 expression might reflect a further predisposition of the Ugandan population to the development of particular types of B-cell neoplasms, such as Waldenström's macroglobulinaemia, which is predominantly LAIR1 negative or comprises both LAIR1-positive and LAIR1-negative B-cell subpopulations, whereas hairy cell leukaemia typically shows strong LAIR1 expression.[22] The use of a hospital-based study population has been shown to have a similar MBL prevalence to population-based studies using a similar screening sensitivity.[5,6] However, the age and sex distribution of the UK study population in our study was selected to match the Ugandan cohort and might therefore under-represent population MBL prevalence. Therefore, further studies of MBL in the general UK population would be informative.

Some data support the case that CD5-negative B-lymphoproliferative disorders are more common than CLL in Africa.[3] The results of our study, with a higher prevalence of CD5-negative MBL than CLL-phenotype MBL, provide further evidence of geographical variation. This finding mirrors the results of Landgren and colleagues' study,[31] which showed that the prevalence and incidence patterns of myeloma are reflected in a much higher incidence of the precursor lesion monoclonal gammopathy of undetermined significance in Africans and African Americans than in Caucasians. Although the use of LAIR1 and CXCR5 expression helps to identify monoclonal or monotypic B-cell expansions, neoplastic populations are difficult to define accurately with only these markers because both markers show heterogeneous—and, for LAIR1, frequently bimodal—expression in non-CLL B-lymphoproliferative disorders. Therefore, we propose that future studies should investigate the distribution of abnormal B-cell expansions by combining the sensitive approach of clonality with LAIR1 expression and a more specific phenotypic analysis—eg, the strong CD25 and weak CD22 expression seen in Waldenström's macroglobulinaemia.[32] This combined approach might help to improve understanding of the potential distribution of B-cell malignancies in the general African population.

The use of an environmentally distinct population in rural Uganda in our study has some advantages for understanding the inherited susceptibility, but a limitation is that the rural Ugandan population might not be representative of the whole population in Uganda. In future studies, we aim to compare the rural and urban populations in Uganda, which would offer insights into the relative contribution of inherited susceptibility and environmental factors to the development of B-cell neoplasms. Although efforts were made to exclude people with immune suppression from this study, we cannot completely rule out the possibility that mild immune modulation was present in some participants. The small numbers of participants in our study relative to some studies in high-income countries might also affect results.

To conclude, the prevalence of MBL is broadly similar in rural Uganda and the UK, but substantial qualitative differences exist, with a lower prevalence of CLL-phenotype MBL and higher prevalence of CD5-negative MBL in the Ugandan cohort than in the UK cohort. These differences are likely to reflect variation in susceptibility alleles and antigenic exposure, which might affect the type of B-lymphoproliferative disorders that become clinically relevant. We believe that the study of MBL is an ideal platform to identify and study apparent differences in B-lymphoproliferative disorders in a way that is independent of health-care provision and referral biases, and to potentially allow validation of putative driver mutations using peripheral blood cells.[33] Additional data are required for confirmation of our findings, and more detailed biological data from patients with symptomatic disease, particularly splenic marginal zone lymphomas, are also needed.

Contributors

ACR and RN designed the study. ACR, AS, RdT, CD, DN, AV, PASE, KS, KW, AK, GA, and RN collected, analysed, and interpreted the data. All authors prepared the report and approved the final version.

Declaration of interests

We declare no competing interests.

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