B cell receptor repertoire profiling reveals distinct clonal architectures and early lymphoma-associated patterns in PPBL

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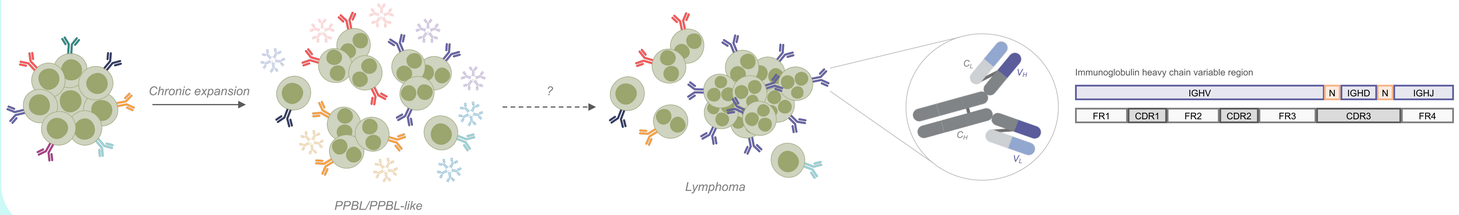
Objective

Persistent polyclonal B cell lymphocytosis (PPBL) is a rare and understudied immune dysregulation, which almost exclusively affects middle-aged women. PPBL is typically characterised by a stable expansion of binucleated marginal-zone-like B cells alongside elevated serum immunoglobulin M (IgM). Despite its generally benign nature, up to 20 % of patients with PPBL will subsequently develop B cell lymphoma, yet molecular predictors of malignant progression remain undefined.
Recently, unbiased hierarchical clustering of B cell-related immune variables within a cohort of patients with primary immune dysregulation, revealed individuals with PPBL-like immune phenotypes, including high polyclonal serum IgM and high percentage of polyclonal marginal zone-like B cells, but these patients lack PPBL diagnosis.
This study aimed to characterise the B cell receptor (BCR) repertoires of PPBL and PPBL-like patients to identify immunogenetic features linked to disease heterogeneity and lymphoma risk.

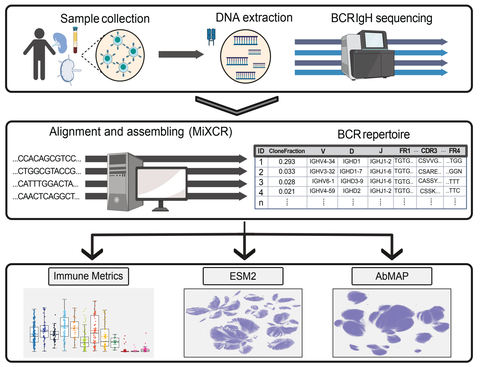


Method

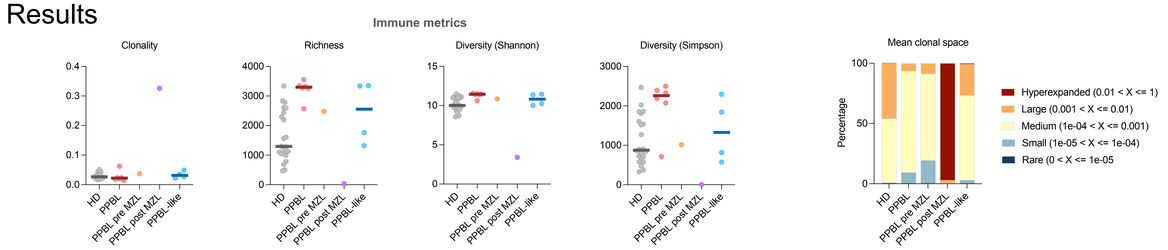
Bulk B cell immunoglobulin heavy chain (IGH) repertoires were sequenced from peripheral blood mononuclear cells of seven PPBL and four PPBL-like patients and compared to age and sex-matched healthy donors (HD).
Reads were processed using standardized pipelines such as MiXCR, TcR, and immunarch to quantify repertoire metrics, clonal space distribution and gene usage. To gain insights into the process of malignant transformation, the BCR repertoire of one PPBL patient who later developed extra-nodal marginal zone B cell lymphoma (MZL) was analysed prior and post diagnosis.

Data

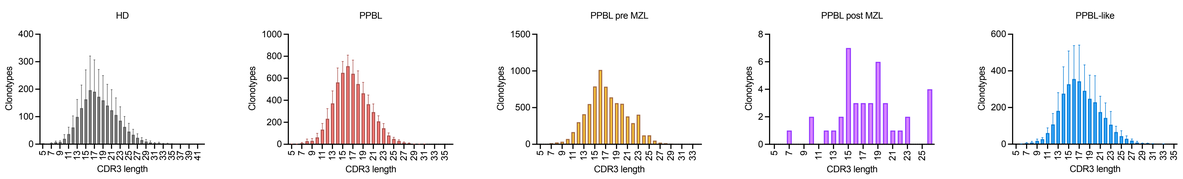
Entity	Samples
HD	22
PPBL	7
PPBL-like	4



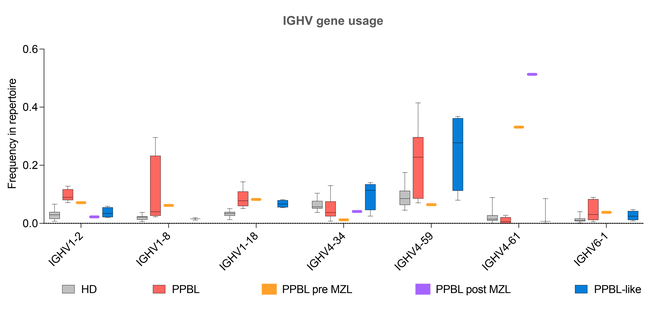
Results

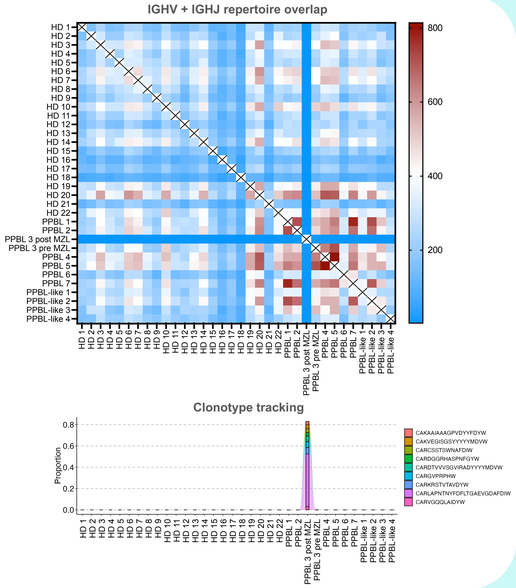


Evaluation of the immune metrics including repertoire clonality, richness, and diversity gives a broad overview of the BCR repertoire (left). All samples demonstrated an evenly distributed, polyclonal repertoire, indicated by low clonality, except for the sample of the PPBL patient diagnosed with MZL. The lymphoma BCR repertoire is highly clonal and consists only of a few unique clonotypes which are highly expanded, highlighted by a low degree of richness and diversity. In this case, almost the entire IGH clonal space is made up of the hyperexpanded malignant clone (right).
In contrast to HDs, BCR repertoires, PPBL patients were found to have a greater number of distinct clonotypes, meaning higher richness. Moreover, the clone frequencies seemed to be better balanced in PPBL samples, indicated by a greater diversity score than HDs. When comparing PPBL to PPBL-like samples, the ladder were also richer and more diverse than the HD repertoires yet not to the same extend as PPBL. Analysis of the clone sizes which make up the mean clonal space (right), demonstrated an increase in small and less large clones for PPBL and PPBL-like patients compared to HDs. No skewing of CDR3 lengths were found for PPBL and PPBL-like samples, except for the PPBL patient after MZL diagnosis.



To investigate whether there were any shared clonotypes between all samples, the IGHV and IGHJ repertoire overlap was determined (upper right). Notably, the most identical sequences were found between PPBL and PPBL-like patients, suggesting some degree of identical BCRs and potential shared epitope targeting. Taking a closer look at the IGHV gene usage (lower), revealed that the PPBL patient had an overrepresentation of IGHV4-61 (30% of repertoire), already three years prior to MZL diagnosis, which then developed into the malignant clone, occupying 50 % of the whole repertoire. Interestingly, prior to MZL development, the patient had a relatively polyclonal BCR repertoire despite the frequent usage of IGHV4-61. Clonotype tracking of the ten most abundant clonotypes after MZL diagnosis revealed that these specific clonotypes were not yet present in the sample of three years prior (lower right). Intriguingly, other PPBL patients also show a preference for certain V genes such as IGHV1-8 or IGHV4-59, making up about one third of their repertoire. When comparing PPBL and PPBL-like samples, the only difference in gene usage was found for IGHV1-2, which is preferred in PPBL patients but not in PPBL-like patients.





Conclusion and outlook

- PPBL and PPBL-like patients show richer and more diverse BCR repertoires than healthy donors, with characteristic preferences for specific IGHV genes.
- IGHV1-2 usage distinguishes PPBL from PPBL-like individuals, while early IGHV4-61 overrepresentation preceded malignant transformation in the PPBL patient who developed MZL.
- Shared clonotype analysis further revealed substantial IGHV/IGHJ overlap between PPBL and PPBL-like patients, suggesting convergent immune responses and potential shared antigenic drivers across these conditions.

Together, these findings provide new insights into the clonal architecture and evolutionary trajectories of BCR repertoires in PPBL and related disorders, and they underscore the potential of IGHV usage patterns as markers of disease subtypes and early malignant transformation. As such, PPBL patients with IGHV gene overrepresentations should be monitored further to determine whether early IGHV biases can help identify patients at risk for clonal expansion and malignant transformation.

References

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