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Finding the five percent: Predicting Adult T-cell Leukaemia/Lymphoma (ATLL) in Human T-cell leukaemia Virus-1 (HTLV-1) carriers using a novel flow cytometric method

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INTRODUCTION

- Adult T-cell leukaemia/lymphoma (ATLL) is an aggressive T cell malignancy caused by the retrovirus Human T-cell leukaemia Virus-1 (HTLV-1) .
- The lifetime incidence in HTLV-1 carriers is 5% but predicting who will transform remains a challenge.
- High Proviral load (PVL, > 4 copies of the HTLV-1 provirus/100 peripheral blood mononuclear cells) is a known predictor of transformation but only 1 in 5 of such patients will transform.
- Infected cells acquiring certain mutations gain a survival advantage resulting in clonal expansion, and potentially ultimately become malignant. Commonly seen mutations in ATLL include those involved in T-cell signalling pathways (e.g. *CCR4, PLCG1*) (1), some of which are seen prior to transformation (2)
- Previous methods for quantifying the unevenness of clone distribution (oligoclonality) have been based on HTLV-1 integration site and required high-throughput sequencing, making them impractical for clinical use.
- We have previously quantified oligoclonality in ATLL using flow cytometry (OCI-flow), by staining infected cells and T cell receptor (TCR) Vβ subunits (3). This is possible as all daughter cells in a clone share the same TCR Vβ subunit. The Oligoclonality Index (OCI) is a measure of frequency distribution where 0 represents a perfectly polyclonal population (each clone present at equal frequency) and 1 represents a perfectly monoclonal population. This scale will allow us to set a threshold to determine those at higher risk of ATLL based on cohorts with known outcome.

AIMS

- To find a simple and reliable method to identify HTLV-1 carriers at the highest risk of transformation.
- To assess additional risk factors that may predict HTLV-1 carriers with a high OCI.
- To assess the mutational and transcriptional profile of the expanded clone in the subsequent phase of this work.

METHOD

- Samples were taken from HTLV-1 carriers attending the National Centre for Human Retrovirology. Written informed consent was obtained and research was conducted under the governance of the Communicable Diseases Research Group Tissue Bank, approved by the UK National Research Ethics Service (09/H0606/106, 15/SC/0089). Peripheral blood mononuclear cells were isolated by density-gradient centrifugation, and stored at -150 °C.
- Thawed cells were stained with an antibody mix including TCR vβ antibody panel covering ~70% of the human TCR repertoire.
- Oligoclonality Index by flow (OCI-flow) was calculated using an established formula based on the Gini index,
- In CD4+ cells, CCR4+CD26- was used as a marker of infected cells, and the most expanded clonal population identified (vβ+). CD7 and Ki-67 expression was also quantified.

RESULTS

- 106 HTLV-1 carriers were screened, of whom 73 (79%) had a high PVL.
- 30 HTLV-1 carriers that had not transformed (median follow-up 10 years) were screened and defined as the 'No ATLL' cohort. 38 ATLL patients were also screened.
- Median OCI-flow was 0.688 in No ATLL cohort, 0.997 in the ATLL cohort (acute/chronic) and 0.692 in the Screening cohort (Fig. 1)
- A threshold was set at 0.770 based on reporter operated curve analysis of the ATLL and No ATLL groups..
- 14 subjects in the screening cohort had an OCI-flow above the threshold ('High OCI'). All subjects had a PVL >4 (19% of high PVL cohort).
- 2 High OCI subjects transformed to ATLL, 41 and 69 months after sample was taken (both acute subtype).
- Subjects in the 'High OCI' group were significantly more likely to be younger, have a family history of ATLL, and have a higher PVL and lymphocyte count (Table 1).
- Analysis of the clonal populations in High OCI subjects showed that CD4+CCR4+CD26- ('infected') cell populations have significantly lower CD7 expression and significantly higher Ki-67 expression than CCR4-/CCR4+CD26- populations.
- However, there was no significant difference in CD7 expression or Ki-67 expression between the ATLL-like clone (vβ+) and the other CCR4+CD26- populations (vβ-)

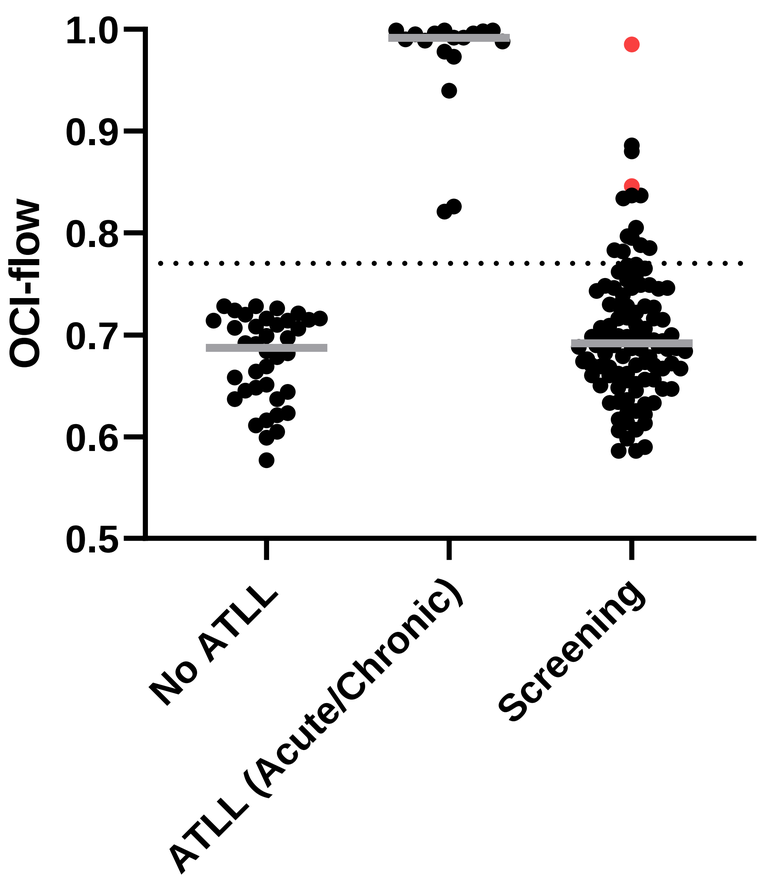

Figure 1. **Oligoclonality index-flow (OCI-flow) score of each cohort**. Samples from a 'No ATLL' (*n*=38), ATLL cohort (*n*=30) and a screening cohort (*n*=132) were stained with a flow panel and OCI-flow calculated mathematically. Statistical analysis was performed using the Kruskal-Wallis test.). ATLL subjects included in receiver operated curve analysis had acute/chronic subtype ATLL (n=17) as disease was primarily in the haematogenous component. Red indicates subjects who subsequently transformed to ATLL.

	High OCI (>0.770)	Low OCI (≤0.770)	p
Age (median, (IQR))	**50 (40-57)**	**57 (48-66)**	**0.04**
Sex (m:f)	36: 64	26: 74	0.52
Ethnicity ('high risk')	100	89	0.6
Immunosuppresive Rx	14%	16%	>0.99
Family history of ATLL	**36%**	**9%**	**0.01**
Proviral load (%, median)	**16.3**	**7.0**	**0.0001**
Lymphocyte count (x 10^9/l, median)	**2.5**	**1.9**	**0.005**

Table 1. Demographic and clinical data from the screening group, subdivided by OCI-flow score. Statistical analysis was performed using Fisher's exact test for categorical variables and Mann-Whitney for continuous variables

CONCLUSIONS

- Expanded clonal populations can be identified quickly and easily in HTLV-1 carriers using this novel flow cytometry method, from 24 hours.
- Around 19% of high PVL HTLV-1 carriers have a 'High OCI', which may represent pre-malignant transformation to ATLL. These subjects show different clinical and haematological features to 'Low OCI' subjects.
- Immunophenotypic markers seen in ATLL, such as CD7 downregulation and Ki-67 expression, are not identified exclusively in expanded clones at this stage, which also suggests that they are pre-malignant at this stage.
- Further work will explore clonal mutations and gene expression in this cohort with a view to identifying those at the highest risk of transformation to ATLL.

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