Non-tumour Bone Marrow Lymphocytes Correlate with Improved Overall Survival in Childhood Acute Lymphoblastic Leukaemia

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Non-tumour Bone Marrow Lymphocytes Correlate with Improved Overall Survival in Childhood Acute Lymphoblastic Leukaemia
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Bone marrow lymphocytes and survival in childhood ALL

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Paediatric cancer, acute lymphoblastic leukaemia, immunophenotype, lymphocyte, overall survival

Abbreviations
ALL    Acute lymphoblastic leukaemia
BM     Bone marrow
CD     Cluster of differentiation
IQR    Inter-quartile range
LFS    Leukaemia free survival
MHC    Major histocompatibility complex
NK     Natural killer
OS     Overall survival
ROC    Receiver operating characteristic
WCC    White cell count
Abstract

Composition of tumour immune cell infiltrates correlate with response to treatment and overall survival (OS) in several cancer settings. We retrospectively examined immune cells present in diagnostic bone marrow aspirate from paediatric patients with B-cell acute lymphoblastic leukaemia. Our analysis identified a sub-group (~30% of patients) with >2.37% CD20 and >6.05% CD7 expression that had 100% OS, and a sub-group (~30% of patients) with ≤2.37% CD20 and ≤6.05% CD7 expression at increased risk of treatment failure (66.7% OS, p<0.05). Immune cell infiltrate at diagnosis may predict treatment response and could provide a means to enhance immediate treatment risk stratification.

Introduction

Conventional chemotherapeutic regimens cure a significant proportion of paediatric B cell acute lymphoblastic leukaemia (B-ALL) patients. Improvements in survival rates observed over recent decades can be attributed to the introduction of efficient molecular testing for minimal residual disease during treatment, and new combinations of chemotherapeutics. However, considerable adverse effect profiles are associated with regimens used to treat paediatric B-ALL. Strategies to identify patients where lower dose treatment would be clinically effective or treatment intensification would enhance survival, represent attractive avenues to reduce long term cytotoxicity and/or enhance overall survival (OS).

Recent advances in our understanding of tumour immunology suggest that an individual’s immune response, pre-therapy against their own tumour, significantly influences disease progression.[1] Several parameters associated with the composition of immune infiltrate in solid tumours have been shown to correlate with prognosis, and in some instances, to predict patient survival more accurately than any other parameter.[2,3] In B-ALL, two recent reports examining
the immunological composition of bone marrow (BM) at diagnosis demonstrate correlation between CD4+ T lymphocytes and favourable early response in paediatric patients[4] and CD8+ T lymphocytes and improved OS in adult patients.[5] In this study, we examined the composition at diagnosis, of non-malignant lymphocytes in the BM of paediatric B-ALL patients. We aimed to identify whether non-malignant lymphocytes routinely measured in BM aspirate by flow cytometry are associated with OS.

Methods & Results

We reviewed the medical records of 153 children diagnosed with ALL at Alder Hey Children’s Hospital, Liverpool between 2002 and 2009 in accordance with NHS Health Research Authority and Royal College of Pathologists’ guidelines. All patients had been treated in accordance with UKALL 97/99 or UKALL 2003 trial protocol. In 55 cases, flow cytometry data from clinical diagnostic BM aspirate could be recovered in List Mode data format and re-analysed to enumerate data related to the non-tumour cells present. All 55 patients analysed were diagnosed with common ALL (de novo precursor B-ALL), patient characteristics are show in Table I. Patients with Philadelphia chromosome positive B-ALL, common ALL with aberrant CD20 tumour expression or Down syndrome were excluded from this study. There were 8 deaths and the remaining 47 patients were alive and well at the time of last follow up, although 4 had experienced relapse, but were treated successfully with salvage chemotherapy.

We assessed the measured relative frequency values of CD markers in diagnostic BM aspirate between survivors and non-survivors. Tumour lymphocytes (CD19+ CD10+) and non-malignant B-lymphocytes (CD19+ CD10-) were equivalent between study groups however, an increased relative frequency of the mature B lymphocyte marker, CD20, was observed in survivors ($p=0.0295$; Figure 1A). T lineage cells (CD2+) were equivalent between study groups however,
an increased relative frequency of the mature T lymphocyte and NK cell marker, CD7, was
observed in survivors ($p=0.0447$; Figure 1B). Following associated Receiver Operating
Characteristic (ROC) analysis, cut-offs of 2.37% CD20 expression (sensitivity 88%, specificity
56%) and 6.05% CD7 expression (sensitivity 88%, specificity 53%) were selected to
differentiate between patients with high and low expression. Comparing patients with high
CD20 expression ($>2.37%; \ n=28$) to those with lower expression ($\leq 2.37%; \ n=27$) we observed
significantly increased OS of 96.4% compared to 74.1% ($p=0.030$; Figure 1C), and improved
leukaemia free survival (LFS) of 92.9% compared to 66.7% ($p=0.024$; Figure 1D). In a similar
analysis of CD7, we observed increased OS of 96.0% in patients with high CD7 expression
($>6.05%; \ n=25$) compared to 76.7% in those with low expression ($\leq 6.05%; \ n=30; \ p=0.041$;
Figure 1E) although no improvement in LFS was noted (Figure 1F). Combining these factors,
we observed patients with high CD20 expression and high CD7 expression (n=16), had 100%
OS compared to 66.7% in patients with low expression of both markers (n=18; Figure 1G;
$p=0.013$), and improved LFS of 87.5% compared to 61.1% ($p=0.045$; Figure 1H).

Discussion

Our retrospective analysis of CD marker expression in diagnostic BM aspirate identified a group
of patients (16 out of 55), that could be characterised at diagnosis according to high CD20 and
high CD7 expression, that experienced 100% OS. Conversely, patients that could be
characterised according to low CD20 and low CD7 expression (18 out of 55) experienced
significantly reduced OS, 66.7%, suggesting that the BM immune infiltrate at diagnosis is
indicative of, or can be correlated with, their response to treatment.

An increasing body of literature exists linking immune system parameters at diagnosis with
prognosis in cancer patients.[1] Immune cell infiltrate may represent an ongoing but ineffective
antitumour immune response, or a collection of tumour promoting cells recruited into the tumour
microenvironment. This so called ‘tumour immune contexture’ may be relevant to
understanding a patients’ response to treatment[6] and the induction of an antitumour immune
response has the potential to enhance survival prospects. Lymphocytes perform immune
surveillance and may recognise malignant cells as immunogenic.[7] CD20 is expressed on
mature B cells with the exception of terminally differentiated plasmablasts or plasma cells.
Resting B cells express surface Ig as a receptor,[8] antigen engagement of surface Ig can activate
B cells, and leads to internalisation of antigen and presentation of peptides complexed with MHC
class II at the cell surface for interaction with CD4+ T cells.[8,9] Lymphocytes are also immune
effector cells, and may mediate tumour cell death through granule exocytosis or death receptor
signalling, cytotoxic T cells and NKs are key effectors in these mechanisms. Interaction between
B and T cells serves to augment adaptive immune responses through cross-priming of T cells,
reciprocal enhancement of activation signalling in each cell type, and the production of
immunostimulatory cytokines.[9,10] Thus, the collaboration of different types of lymphocyte
may confer the capacity to develop a robust adaptive immune response against autologous
tumour cells.

Our findings, combined with those reported by Lustfeld et al. (2014), provide the first
information as to the relevance of the tumour immune contexture in paediatric ALL. An
increased frequency of mature immune cell components within the BM tumour
microenvironment is strongly associated with both early treatment response[4] and successful
chemotherapeutic treatment. Further, elucidating the relationships between immune infiltrate
components at diagnosis and treatment response may provide a means to enhance immediate
treatment risk stratification. Of the 8 children that died in this study, 3 had no clinical high risk
features and were treated according to Regimen A. All 3 of these children had low CD20 expression levels at diagnosis, and 2 of these 3 also had low CD7 expression levels. Reliable identification of very good risk, and very high risk patients at diagnosis potentially offers the opportunity to reduce or intensify therapy from induction onward. Our data clearly define two important patient sub-groups; firstly one for which the chemotherapeutic treatment regimen was 100% successful, and secondly one where patients were at significantly increased risk of treatment failure. Notwithstanding the limitations of this single centre retrospective study of small numbers, it seems relevant that the tumour immune contexture in paediatric ALL receive future attention. Both to confirm our observations and to investigate mechanistically, the benefit to patients of mature immune cell BM infiltrate at treatment commencement.

Acknowledgements

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Conflict of interest

The authors declare they have no competing interests.

References


Brief report


(10) Clark EA, Ledbetter JA. How B-cells and T-cells talk to each other. Nature
Legends

Figure 1. CD20+ lymphocytes were increased in survivors compared to non-survivors (A), CD7+ lymphocytes were increased in survivors compared to non-survivors (B). Enhanced OS (C) and LFS (D) was observed in paediatric B-ALL patients with >2.37% CD20+ lymphocytes. Enhanced OS (E) but not LFS (F) was observed in paediatric B-ALL patients with >6.05% CD7+ lymphocytes. 100% OS was observed in patients with >2.37% CD20+ and >6.05% CD7+ lymphocytes (G). 87.5% LFS was observed in patients with >2.37% CD20+ and >6.05% CD7+ lymphocytes compared to 61.1% LFS in patients with ≤2.37% CD20 and ≤6.05% CD7 expression (H).
Table I. Patient Characteristics

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<th>Study cohort n=55</th>
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<tr>
<td>Median age in years, n (IQR)</td>
<td>5.0 (2.5 - 7.5)</td>
<td>4.0 (1.5 - 6.5)</td>
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<tr>
<td>Male gender, n (%)</td>
<td>30 (54.5)</td>
<td>83 (54.2)</td>
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<tr>
<td>Age groups:</td>
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<tr>
<td>&lt;10 years, n (%)</td>
<td>44 (80.0)</td>
<td>125 (81.7)</td>
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<tr>
<td>≥10 years, n (%)</td>
<td>11 (20.0)</td>
<td>28 (18.3)</td>
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<td>WCC at diagnosis:</td>
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<tr>
<td>&lt;50 x 10^9 L^{-1}, n (%)</td>
<td>46 (83.6)</td>
<td>129 (84.3)</td>
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<tr>
<td>≥50 x 10^9 L^{-1}, n (%)</td>
<td>9 (16.4)</td>
<td>24 (15.7)</td>
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<td>Initial treatment protocol:</td>
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<tr>
<td>Regimen A (low risk), n (%)</td>
<td>36 (65.5)</td>
<td>103 (67.3)</td>
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<tr>
<td>Regimen B (high risk), n (%)</td>
<td>19 (34.5)</td>
<td>50 (32.7)</td>
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IQR, inter-quartile range; WCC, white cell count.
Figure 1. CD20+ lymphocytes were increased in survivors compared to non-survivors (A), CD7+ lymphocytes were increased in survivors compared to non-survivors (B). Enhanced OS (C) and LFS (D) was observed in paediatric B-ALL patients with >2.37% CD20+ lymphocytes. Enhanced OS (E) but not LFS (F) was observed in paediatric B-ALL patients with >6.05% CD7+ lymphocytes. 100% OS was observed in patients with >2.37% CD20+ and >6.05% CD7+ lymphocytes (G). 87.5% LFS was observed in patients with >2.37% CD20+ and >6.05% CD7+ lymphocytes compared to 61.1% LFS in patients with ≤2.37% CD20 and ≤6.05% CD7 expression (H).