IMMUNOPHENOTYPIC CHARACTERISATION AND EVALUATION OF MINIMAL RESIDUAL DISEASE (MRD) IN ACUTE LEUKAEMIA PATIENTS IN SARAWAK BY FLOW CYTOMETRY

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ABSTRACT

Leukaemia is a malignant disease of the bone marrow and blood which usually affect children and adults. Minimal residual disease (MRD) is the definition given to a small numbers of leukaemic cells that remain in the patient when the patient is in remission, and a known cause of relapse in cancer and leukaemia. Using immunophenotypic multiparametric flow cytometry, sequential studies (diagnosis and follow-up) of the characterisation of the leukaemic blast and the association between the clinical parameters of 147 acute myeloid leukaemia (AML) patients and 122 acute lymphoblastic leukaemia (ALL) were investigated. Patients diagnosed with acute leukaemia were recruited from hospitals all over Sarawak within the duration of 3 years, from 2007 until 2010. Patients were studied at diagnosis with a panel of monoclonal antibodies in 3- and 4-colour antibody combinations for detections of common, aberrant or uncommon phenotypic features. Using the patient’s immunophenotypic features during diagnosis, the identification of residual leukaemic cells were made possible after completion of induction chemotherapy and the frequency of antigen expression was determined for minimal residual detection. Other clinical data such as haemoglobin level, lymph node enlargement, liver, splenomegaly, platelet and white blood count were also analysed. The odds ratio between MRD and the relevant parameters involved was also calculated with statistical software. The data were analysed statistically and \( p \) value < 0.05 is considered as statistically significant. In the AML cases, female childhood patients were found to have higher total white count compared to males \( (p =0.008) \). Immunophenotypic results also showed similar expressions between childhood and adult group with high expression of CD33, CD13 and aberrant marker such as CD19, and may be established as diagnosis standard markers. In the ALL cases, for the Malay/Melanau ethnic
group, more adult males were diagnosed with B-ALL compared to adult females ($p=0.043$). Antigen expressions for both childhood and adult groups were similar to previous studies, with CyCD79a, CD19, CD22 for B-ALL, while CD7 and CyCD3 for T-ALL, thus suggesting the antibody panels used may be established as diagnostic markers. For the MRD analysis, only 28 AML cases (19.0%) were analysed for MRD and no clinical and immunophenotypic parameter was significantly related with MRD outcome. Multiple logistic regression (MLR) analysis for AML was also not significant. As for ALL, 77 cases (64.2%) were studied for MRD. It was found that low haemoglobin level was significantly associated with positive MRD ($p<0.001^*$). In MLR analysis, haemoglobin level and splenomegaly were found to be significantly associated with MRD positivity, with odd risks of 1.68 and 37.98 respectively. Overall, from the study it can be concluded that clinical parameters and immunophenotypic expressions could be used as a predictive factors in MRD outcome. This study could be improved by a greater effort to coordinate leukaemia patients’ treatment and follow up in Sarawak. A more comprehensive study regarding MRD in acute leukaemia patients is therefore recommended in order to provide the health providers more concrete data about the prognostic/predictive factors that may influence the disease outcome, and eventually assist in the future treatment and management of acute leukaemia in Sarawak.
ABSTRAK

Leukemia adalah penyakit malignant bagi tulang sumsum dan darah yang selalunya melibatkan golongan kanak-kanak dan dewasa. Penyakit residu minimal (MRD) boleh diterangkan sebagai sebahagian kecil sel leukemia yang masih tertinggal di dalam pesakit semasa pesakit berada dalam keadaan remisi, dan dikenalpasti sebagai punca utama leukemia dan kanser berulang.

untuk kumpulan pesakit AML dewasa dan kanak-kanak, khasnya ekspresi yang tinggi bagi CD33, CD13 dan juga penanda aberrant seperti CD19, dan boleh digunakan sebagai penanda standard bagi kes diagnosis. Hasil kajian kes bagi diagnosis ALL pula mendapati bahawa dalam etnik Melayu/Melanau, lelaki dewasa lebih cenderung didiagnosis dengan sub-jenis ALL iaitu B-ALL berbanding wanita dewasa ($p = 0.043$). Ekspresi antigen untuk kedua kumpulan kanak-kanak dan dewasa juga menunjukkan persamaan ekspresi yang hampir sama, dengan ekspresi tinggi bagi CyCD79a, CD19, CD22 bagi B-ALL manakala CD7 dan CyCD3 untuk sub-jenis T-ALL. Bagi kajian MRD, hanya sebanyak 28 kes AML (19.0%) daripada kes-kes diagnosis telah dianalisis untuk MRD dan tiada parameter klinikal dan immunophenotypic yang dijumpai signifikan dengan hasil MRD. Analisis multiple logistic regression (MLR) bagi AML juga didapati tidak signifikan dengan MRD. Bagi ALL, sebanyak 77 kes (64.2%) daripada kes-kes diagnosis elah layak diteruskan untuk analisis MRD. Didapati bahawa tahap haemoglobin yang rendah bagi pesakit ALL adalah signifikan dengan MRD yang positif ($p<0.001$*). Bagi analisis MLR pula, tahap hemoglobin dan splenomegaly didapati mempunyai perkaitan yang signifikan dengan hasil MRD yang positif, dengan masing-masing mempunyai nisbah risiko sebanyak 1.68 dan 37.98. Keseluruhan daripada kajian ini dapat disimpulkan bahawa parameter-parameter klinikal dan ekspresi immunophenotypic boleh diambil kira sebagai faktor ramalan bagi hasil MRD. Kajian ini boleh ditambah baik dengan usaha yang lebih berkesan dan menyeluruh dalam mengkoordinasi rawatan pesakit leukemia serta rawatan susulan di Sarawak khasnya. Kajian yang lebih komprehensif berkenaan MRD di Malaysia pula adalah disyorkan untuk menyediakan data yang lebih konkrit berkenaan faktor prognostik/ramalan yang boleh mempengaruhi prognosis penyakit kepada penyedia penjagaan kesihatan, sekalgus membantu mereka dalam rawatan dan pengurusan pesakit yang lebih berkesan pada masa hadapan.
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Figure 1.5.3 The dot plots (a)-(l) from a patient positive for childhood T-ALL. These plots were taken from the T-ALL immunophenotyping panel performed on the sample. The blast populations (in pink) were positive for the following antigens: CD34, CD45, CD56, CD10, CyCD79a, CD8, nTdT, CyCD3, CD3, CD7 and CD5.

Figure 1.5.4 The distribution of B-ALL, T-ALL in adult ALL

Figure 1.5.5 The age distribution of adult ALL cases

Figure 1.5.6 The sex distribution of adult ALL cases

Figure 1.5.7 Ethnicity distribution of adult ALL cases in Sarawak

Figure 1.5.8 These were the dot plots of the patient positive for adult B-ALL. These plots were taken from the acute leukaemia screening and B-ALL immunophenotyping panel performed on the sample. The blast populations (in pink) were positive for the following antigens: CD34.

Figure 1.5.9 The plots (a) to (h) were taken from a patient positive for adult T-ALL. The plots were taken from the acute leukaemia screening and T-ALL immunophenotyping panel performed on the sample. The blast populations (in pink) were positive for the following antigens: CD45, CD34, nTdT, CD5, CyCD3 and CD7.

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