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CLINICAL EVALUATION AND PROTEOMIC PROFILES OF ACUTE MYELOID LEUKEMIA PATIENTS, SAUDI ARABIA

ALMAIMAN AMER ABDULRAHMAN

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By

ALMAIMAN AMER ABDULRAHMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements of the Degree of Doctor of Philosophy

December 2015
DEDICATIONS

Dedicate to my father, my mother and my wife
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

CLINICAL EVALUATION AND PROTEOMIC PROFILES OF ACUTE MYELOID LEUKEMIA PATIENTS, SAUDI ARABIA

By

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December 2015

Chairman: Prof. Rasedee Abdullah, PhD
Faculty: Institute of Biosciences

Acute myeloid leukemia (AML) represents a group of clonal hematopoietic stem cell disorders which fail to differentiate and proliferate result in the accumulation of non-functional myeloblasts. In AML, response of patients to therapy is variables and there is no reliable marker to predict treatment response or prognosis of the disease. In this study, the main objective was to determine proteomic profiles of AML in patients referred to the King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia. The AML patients were categorized according to risk degree based on the probability of survival. Clinical data were collected from 22 patients (16 males and 6 females) with consent. A clinical data form was developed to collect information on the diagnosis, prevention, and follow-up monitoring of AML patients.

The clinical data showed that most patients (14) were cytogenetically and genetically normal and classified under the intermediate-risk category. Six patients expressed complex CCAAT/enhancer binding protein (CEBPA), FMS-related tyrosine kinase (FLT), isocitrate dehydrogenase (IDH) and Wilms tumor 1 (WT1) gene abnormalities, 2 patients showed inversion at chromosome 16 (inv.16) and 1 had t(15:17) chromosomal translocation.
The high abundance protein in the samples must be depleted before subjecting to proteomic analyses. In the current study, the Pierce Albumin and IgG Removal, Albumin Depletion, and ProteoPrep® Immunoaffinity Albumin and IgG Depletion Kits were compared in the optimization of the samples. The Pierce Albumin and IgG Removal Kit was found to be the best depletion method for high abundance proteins.

The protein expressions were compared among patients with different risk categories, at diagnosis and after remission. The AML samples from peripheral blood and bone marrow were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). At diagnosis, in both the peripheral blood and bone marrow samples, 21 proteins were significantly (\( P<0.05 \)) different in differential expressions among groups. The total number of proteins detected in peripheral blood was similar to those detected in bone marrow. However, there were differences in proteomic profiles between samples collected at diagnosis and remission. Most differentially expressed proteins were down-regulated in peripheral blood at remission. Among the AML cases in this study, 144 differentially expressed proteins were identified, and differences in their expression levels correlated with AML risk categories. The study showed that amphiregulin precursor (AR) (colorectum cell-derived) was expressed in low-risk AML patients only, while haptoglobin α and β and cytoplasmic tyrosyl-tRNA synthetase (EC 6.1.1.1) were highly expressed in intermediate-risk AML patients only. Lysine-arginine-ornithine-binding periplasmic protein (LAO) was expressed in intermediate and high-risk and not in the low-risk AML patients.

In conclusion, the study showed that complete clinical data are essential for the determination of correlation between proteomic profile, risk categories and prediction of response to chemotherapy in AML patients. AR, haptoglobin α and β and LAO may serve as potential biomarkers for the determination of risk categories and responsiveness of AML patients to chemotherapy.

**Keywords:** Acute myeloid leukemia, risk categories, proteomics.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENILAIAN KLINIKAL DAN PROFIL PROTEOMIK LEUKEMIA MIELOID AKUT PESAKIT, ARAB SAUDI

Oleh

ALMAIMAN AMER ABDULRAHMAN

Disember 2015

Pengerusi: Prof. Rasedee Abdullah, PhD
Fakulti: Institut Biosains

Leukemia mieloid akut (AML) mewakili sekumpulan gangguan sel dasar hematopoiesis berklon di mana kegagalan untuk membeza dan memproliferat mengakibatkan terkumpulnya mieloblas yang tidak berfungsi. Dalam AML, gerak balas pesakit terhadap terapi adalah berbeza dan tiada satu penanda yang betul boleh dipercayai untuk meramal gerak balas terhadap rawatan atau prognosis penyakit ini. Dalam kajian ini, objektif utama adalah menentukan profil proteomik AML untuk pesakit yang dirujuk kepada King Faisal Specialist Hospital and Research Center, Riyadh, Arab Saudi. Pesakit AML dikategorikan mengikut tahap risiko berasaskan kemungkinan mandiri. Data klinikal telah dikumpulkan daripada 22 pesakit (16 lelaki dan 6 perempuan) dengan izin. Borang data klinikal telah diwujudkan mengumpul keterangan mengenai diagnosis, pencegahan, dan pemantauan susulan serta pesakit AML.

Data klinikal menunjukkan bahawa kebanyakkan pesakit (14) adalah normal sitogenetik dan genetiknya dan diklasifikasi di bawah kategori risiko sederhana. Enam pesakit menyatakan kompleks protein pengikat CCAAT/peningkat (CEBPA), tirosine kinase terkait-FMS (FLT), isositrat dehidrogenase (IDH) dan keabnormalan gen tumor Wilms 1 (WT1), 2 menunjukkan pembalikan pada kromosom 16 (inv.16) dan 1 dengan translokasi kromosom t(15:17).
Protein berlebihan tinggi dalam sampel mesti disingkirkan sebelum perlakuan analisis proteomik. Dalam kajian ini, tiga kaedah untuk pengoptimuman sampel, iaitu kit Pierce Albumin and IgG Removal, Albumin Depletion, and ProteoPrep® Immunoaffinity Albumin and IgG Depletion telah dibanding. Kit Pierce Albumin and IgG Removal didapati kaedah yang terbaik untuk penyingkiran protein berlebihan tinggi.

Penyataan protein telah dibandingkan di kalangan pesakit berlainan kategori risikonya, semasa diagnosis dan selepas kebah. Sampel AML daripada darah periferi dan sumsum tulang telah dianalisiskan melalui spektrometri jisim seiring kromatografi ceceair (LC-MS/MS). Pada masa diagnosis, dalam kedu-dua sampel darah periferi dan sumsum tulang, 21 protein didapati berbeza secara tererti \((P<0.05)\) dengan nyata pembezaannya di kalangan kumpulan. Bilangan protein yang dikesan dalam darah periferi sama dengan sumsum tulang. Bagaimanapun, terdapat kelainan dalam profil proteomik antara sampel yang diambil semasa diagnosis dan kebah. Kebanyakan protein nyata pembezaan telah terkawal-turun dalam darah periferi pada masa kebah. Di kalangan kes dalam kajian ini, 144 protein nyata pembezaan dikenal pasti, dan kelainan dalam tahap penyataan berkorelasi dengan kategori risiko AML. Kajian itu menunjukkan bahawa pelopor amfiregulin (AR) (terbitan sel kolorektum) ternyata dalam pesakit AML berisiko rendah sahaja, sementara haptoglobin α dan β dan sintetase tirosil-tRNA sitoplasma (EC 6.1.1.1) ternyata tinggi dalam pesakit AML berisiko sederhana sahaja. Protein periplasma pengikat-lisina-arginina-ornitina (LAO) ternyata dalam pesakit AML yang berisiko sederhana dan tinggi dan bukan dalam yang berisiko rendah.

Kesimpulannya, kajian ini menunjukkan bahawa data klinikal lengkap adalah perlu untuk penentuan korelasi antara profil proteomik, kategori risiko dan ramalan gerak balas terhadap kemoterapi dalam pesakit AML. Pelopor amfiregulin, haptoglobin α dan β dan LAO mungkin boleh bertindak sebagai biopendana berpotensi dalam penentuan kategori risiko dan tahap gerak balas pesakit AML terhadap kemoterapi.

Katakunci: leukemia mieloid akut, kategori risiko, proteomik.
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I certify that a Thesis Examination Committee has met on 8 December 2015 to conduct the final examination of Almamn Amer Abdulrahman A on his thesis entitled "Clinical Evaluation and Proteomic Profiles of Acute Myeloid Leukemia Patients, Saudi Arabia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

**Abd. Wahid bin Haron, PhD**
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

**Md Zuki bin Abu Bakar @ Zakaria, PhD**
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Internal Examiner)

**Arifah binti Abdul Kadir, PhD**
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

**Fang-Rong Chang, PhD**
Professor
Kaohsiung Medical University
Taiwan
(External Examiner)

[Signature]

**ZULKARNAIN ZAINAL, PhD**
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 January 2016
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of doctor of philosophy. The members of the Supervisory Committee were as follows:

**Rasedee Abdullah, PhD**  
Professor  
Faculty of veterinary medicine  
Universiti Putra Malaysia  
(Chairman)

**Ahmad Bustamam Abdul, PhD**  
Professor  
Faculty of medicine and health sciences  
Universiti Putra Malaysia  
(External Member)

**Zeenathul Nazariah Allauddin, PhD**  
Associate Professor  
Faculty of veterinary medicine  
Universiti Putra Malaysia  
(External Member)

**Ayodele A. Alaiya, PhD**  
Professor  
Proteomics Unit  
King Faisal Specialist Hospital and Research Center  
Riyadh, Saudi Arabia  
(External Member)

**Eltayeb E.M. Eid, PhD**  
Assistant Professor  
Pharmacy School, Qassim University  
Qassim, Saudi Arabia  
(External Member)

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______________________________
Signature:

Name of Member of Supervisory Committee: Prof. Ahmad Bustamam Abdullah

______________________________
Signature:

Name of Member of Supervisory Committee: Assoc. Prof. Zeenathul Norziah Allauddin

______________________________
Signature:

Name of Member of Supervisory Committee: Dr. Ayodele A. Alaiya

______________________________
Signature:

Name of Member of Supervisory Committee: Dr. Eltayeb E.M. Eid
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<td>Curve observation of one of the differentially expressed proteins among the different risk categories of acute myeloid leukemia</td>
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<td>5.3</td>
<td>Chromatograms of plasma samples from high risk category of acute myeloid leukemia</td>
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<td>Chromatograms of plasma samples from intermediate risk category of acute myeloid leukemia</td>
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<td>5.5</td>
<td>Chromatograms of plasma samples from low risk category of acute myeloid leukemia</td>
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### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADH</td>
<td>Alcohol dehydrogenase</td>
</tr>
<tr>
<td>ALL</td>
<td>Acute lymphoid leukemia</td>
</tr>
<tr>
<td>AmBic</td>
<td>Ammonium bicarbonate</td>
</tr>
<tr>
<td>AML</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>BM</td>
<td>Bone marrow</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone marrow cell</td>
</tr>
<tr>
<td>BMP</td>
<td>Bone marrow plasma</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete blood count</td>
</tr>
<tr>
<td>CDK</td>
<td>Cyclin-dependent kinase</td>
</tr>
<tr>
<td>CEBPA</td>
<td>CCAAT/enhancer-binding protein</td>
</tr>
<tr>
<td>CHAPS</td>
<td>3[3-(3-Cholamidopropyl) dimethylammonio] propanesulfonic acid</td>
</tr>
<tr>
<td>CLL</td>
<td>Chronic lymphoid leukemia</td>
</tr>
<tr>
<td>CLP</td>
<td>Common lymphoid progenitor</td>
</tr>
<tr>
<td>CML</td>
<td>Chronic myeloid leukemia</td>
</tr>
<tr>
<td>CMP</td>
<td>Common myeloid progenitor</td>
</tr>
<tr>
<td>CN-AML</td>
<td>Normal cytogenetic-acute myeloid leukemia</td>
</tr>
<tr>
<td>CPF</td>
<td>Core binding factor</td>
</tr>
<tr>
<td>CR</td>
<td>Complete remission</td>
</tr>
<tr>
<td>CSF-1</td>
<td>Colony-stimulating factor 1</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton</td>
</tr>
<tr>
<td>DBP</td>
<td>D-binding protein precursor</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>DE</td>
<td>Dimensional electrophoresis</td>
</tr>
<tr>
<td>Del</td>
<td>Deletion</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>ETO</td>
<td>Eight twenty-one proteins</td>
</tr>
<tr>
<td>FAB</td>
<td>French-american-british</td>
</tr>
<tr>
<td>FLT</td>
<td>Fms-like tyrosine</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrogen chloride</td>
</tr>
<tr>
<td>Hct</td>
<td>Hematocrit</td>
</tr>
<tr>
<td>HDMS</td>
<td>High-definition mass spectrometry</td>
</tr>
<tr>
<td>HIC</td>
<td>Hydrophobic interaction chromatography</td>
</tr>
<tr>
<td>HIDAC</td>
<td>High dosage cytarabine</td>
</tr>
<tr>
<td>HAS</td>
<td>Human albumin serum</td>
</tr>
<tr>
<td>HSCs</td>
<td>Hematopoietic stem cells</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>Human T-lymphotropic virus</td>
</tr>
<tr>
<td>IA</td>
<td>Immunoaffinty</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>IAA</td>
<td>Iodoacetamide</td>
</tr>
<tr>
<td>ICE</td>
<td>Ifosfamide, carboplatin, and etoposide</td>
</tr>
<tr>
<td>IDH</td>
<td>Isocitrate dehydrogenase</td>
</tr>
<tr>
<td>IEF</td>
<td>Isoelectric focusing</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>Inv</td>
<td>Inversion</td>
</tr>
<tr>
<td>IPA</td>
<td>Ingenuity pathway analysis</td>
</tr>
<tr>
<td>IPG</td>
<td>Immobilized pH gradient</td>
</tr>
<tr>
<td>LC/MS/MS</td>
<td>Liquid chromatography/tandem mass spectrometry</td>
</tr>
<tr>
<td>LT-HSC</td>
<td>Long-term hematopoietic stem cell</td>
</tr>
<tr>
<td>M1</td>
<td>Acute myeloblastic leukemia with minimal maturation</td>
</tr>
<tr>
<td>M2</td>
<td>Acute myeloblastic leukemia with maturation</td>
</tr>
<tr>
<td>M3</td>
<td>Acute promyelocytic leukemia</td>
</tr>
<tr>
<td>M4</td>
<td>Acute myelomonocytic leukemia</td>
</tr>
<tr>
<td>M5</td>
<td>Acute monocytic leukemia</td>
</tr>
<tr>
<td>M6</td>
<td>Acute erythroid leukemia</td>
</tr>
<tr>
<td>M7</td>
<td>Acute megakaryocyte leukemia</td>
</tr>
<tr>
<td>MaPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MALDI</td>
<td>Matrix-assisted laser desorption ionization</td>
</tr>
<tr>
<td>MDR</td>
<td>Multidrug resistant</td>
</tr>
<tr>
<td>MLL</td>
<td>Multi-lineage leukemia</td>
</tr>
<tr>
<td>MLP</td>
<td>Multiple lymphomatous polyposis</td>
</tr>
<tr>
<td>MPO</td>
<td>Myeloperoxidase</td>
</tr>
<tr>
<td>MRD</td>
<td>Minimal residual disease</td>
</tr>
<tr>
<td>MRN</td>
<td>Medical record number</td>
</tr>
<tr>
<td>MSE</td>
<td>Data-independent acquisition</td>
</tr>
<tr>
<td>Mg</td>
<td>Microgram</td>
</tr>
<tr>
<td>Ml</td>
<td>Microliter</td>
</tr>
<tr>
<td>UPLC</td>
<td>Ultra performance liquid chromatography</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>NK cells</td>
<td>Natural killer cells</td>
</tr>
<tr>
<td>NPM</td>
<td>Nucleophosmin</td>
</tr>
<tr>
<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>PB</td>
<td>Peripheral blood</td>
</tr>
<tr>
<td>PBC</td>
<td>Peripheral blood cell</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
</tr>
<tr>
<td>PBP</td>
<td>Peripheral blood plasmas</td>
</tr>
<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PCA</td>
<td>Principle component analysis</td>
</tr>
<tr>
<td>PH</td>
<td>Partial pressure of (element)</td>
</tr>
<tr>
<td>Plt</td>
<td>Platelet</td>
</tr>
<tr>
<td>PML</td>
<td>Promyelocytic leukemia</td>
</tr>
<tr>
<td>PMSF</td>
<td>Phenylmethylsulfonyl fluoride</td>
</tr>
<tr>
<td>PTM</td>
<td>Post-translational modification</td>
</tr>
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xvi
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>RAR</td>
<td>Retinoic acid receptor</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cell</td>
</tr>
<tr>
<td>RBM</td>
<td>Round per minute</td>
</tr>
<tr>
<td>Rh</td>
<td>Rhesus</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>RUNX1</td>
<td>Runt-related transcription factor 1</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>ST-HSC</td>
<td>Short-term hematopoietic stem cell</td>
</tr>
<tr>
<td>TCR</td>
<td>T-cell antigen receptor</td>
</tr>
<tr>
<td>TET2</td>
<td>Ten eleven translocation 2</td>
</tr>
<tr>
<td>TKD</td>
<td>Tyrosine kinase domain</td>
</tr>
<tr>
<td>TOF</td>
<td>Time-of-flight</td>
</tr>
<tr>
<td>Tris</td>
<td>Tris (hydroxymethyl) aminomethane</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cell</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organization</td>
</tr>
<tr>
<td>WT1</td>
<td>Wilms tumor 1</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.0 Leukemia and proteomics

Acute and chronic hematological malignancies or “blood cancers” form a distinct subset of cancers that originate in the bone marrow or lymph nodes (Aquino, 2002; Wartenberg et al., 2008). Leukemia is a group of disorders characterized by the accumulation of abnormal leukocytes in the bone marrow and blood circulation. The occupation of these abnormal cells may cause bone marrow failure (Lane and Gilliland, 2010).

Leukemogenesis is a multistep process with several factors that could influence normal hematopoiesis, including over-expression of certain oncogenes, aberrant intracellular pathways, behavior of oncoproteins and expression of chromosomes (Reikvam et al., 2011). Acute myeloid leukemia (AML) is a hematological malignancy characterized by clonal proliferation of immature myeloid precursors and arrest in the maturation of these cells (Rubnitz et al., 2010). These abnormalities cause accumulation of leukemic blast cells in the bone marrow and eventually in peripheral blood. The bone marrow infiltration by the immature cells causes a decrease in blood cell production and thereby reducing the peripheral blood numbers of mature erythrocytes (RBCs), leucocytes (WBCs) and platelets (Plts), causing anemia, thrombocytopenia that eventually lead to hemorrhage, neutropenia and loss of resistance to infections. Thus, in AML, patients are initially presented with symptoms often related to pancytopenia, anemia, weakness, fatigue, infections and/or bleeding (Hamid, 2013).

The incidence of AML is about five cases per 100,000 individuals annually and it accounts for approximately 80% of acute leukemias in adults and 15 to 20% in children (Robison et al., 2002; Deschler and Lübbert, 2006; Chen et al., 2014). In infants, leukemia is unique in epidemiological, biological, and clinical characteristics. Most of the acute lymphoblastic leukemia (ALL) and AML cases in infants are characterized by high leukocyte count, extramedullary disease, propensity for expression of lymphoid and myeloid phenotypic markers, and translocation of the mixed-lineage leukemia (MLL) (ALL-1, HRX, Htrx-1) gene at chromosome band 11q23 (Reikvam et al., 2011), rapid tumor cell proliferation and a predominance of blast cells. Acute lymphoblastic leukemia is the most common form of leukemia which accounts for 75 to 80% of all acute leukemia cases in children and adolescents, while AML accounts for 15 to 20% (Yeates et al., 2009). With modern intensive chemotherapy and supportive care, the prognosis of ALL and to a lesser extent AML in children has improved significantly over the past decades (Shipley and Butera, 2009).
The majority of leukemia studies focused on the correlation between leukemic cell phenotypes with cytogenetic and clinical response with the induction and consolidation therapies. Others have evaluated clinical outcomes of different AML subtypes. Few studies have demonstrated the potential of proteomics in the characterization, diagnosis and treatment of hematological malignancies (Luczak et al., 2012). The weakness of leukemia studies lies in the relatively low sample size and more importantly, in the lack of validation of results and with no follow-up pilot clinical trial on the discoveries from their studies.

Currently, apart from the clinical evaluation coupled with hematological, cytogenetic and molecular tests to monitor patient's responses, there is no available objective protein marker for accurate prediction and monitoring of treatment response in AML. There is therefore a need to develop protein markers that could be complementary to currently diagnostic tools in the management of AML patients.

Recent advancements in proteomic technologies have generated vast interest in the pursuit for biomarkers for various hematological malignancies. Large-scale analysis of proteins from cells, tissues, fluids, or organs, using the classical proteomic platforms has yielded information on the disease heterogeneity at different stages of development and progression. Global differential protein profiling analyses or expression proteomics have resulted in identification of disease-related or tissue-specific proteins that could potentially be useful as disease biomarkers (Alaiya et al., 2011).

This quest to translate basic discoveries into patient care regimes has resulted in many proteomic studies describing potential biomarkers. It is disappointing that despite the technological advances, very few studies have found way into clinical trials. The majority of the published proteomic studies have limitations, including inadequate sample representation, non-standardized sample handling and processing, and extreme dynamic range of serum proteins and above the modest sample size, which does not accurately represent the clinical cohorts (Eun et al., 2004; Visanji et al., 2012). It is therefore important that polypeptides identified as potential biomarkers are validated in appropriately processed, well-characterized clinical samples using established techniques, such as immunochemistry or PCR-based methods (Pitkanen and Lukasiuk, 2011).

The understanding of the molecular mechanisms in treatment responses and disease recurrence is limited in AML. Proteomics is the logical approach because proteins change with cellular responses to internal environment, external stimuli, and disease development.
The majority of available biomarkers are single biomolecules with low sensitivity and specificity. Therefore, the use of a combination of measurable protein panels for a more accurate diagnosis and prediction of disease prognosis in AML.

1.1 Hypothesis

Proteomic profiling in combination with clinical data would support the diagnosis, prediction of progression, and risk categorization of acute myeloid leukemia.

1.2 Problem statement

Cancer is among the main causes of death due to disease in developed countries and developing countries (Siegel et al., 2014). Several studies have demonstrated the potential of proteomics and clinical data in the characterization, diagnosis, and treatment of AML (Luczak et al., 2012). However, to date no protein marker has been identified that can accurately be used in the prediction and determination of treatment response in AML. The discovery of AML diagnostic protein markers will complement the currently available diagnostic tools in the management of AML patients.

1.3 General objective

The general goal of this study was to evaluate the AML risk categories and correlate their global protein expressions with the response to induction and consolidation treatments and overall outcome of the disease.

1.4 Specific objectives of the study

The specific objectives of the study are to:

1. Evaluate the clinical data of acute myeloid leukemia.
2. Correlate the proteins fingerprinting with clinical data of AML patients.
3. Optimize high abundance plasma protein removal for accurate and reproducible proteomic analysis in AML.
4. Determine the proteomic profiles of AML using Synapt G2 platform.
REFERENCES


specific incidences of cytogenetic subgroups of acute myeloid leukemia. 

*Hematologica*, 90(11): 1502-1510.


Mahn, A., Reyes, A., Zamorano, M., Cifuentes, W. & Ismail, M. (2010). Depletion of highly abundant proteins in blood plasma by hydrophobic interaction


