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Review

Current advances in mass spectrometry-based proteomic studies on childhood acute lymphoblastic leukemia

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ABSTRACT

Pediatric cancers are a group of diseases of genetic origin that represent the first cause of death in the pediatric population in developed countries. Particularly, acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood. Correct diagnosis of ALL is of vital importance for the success of the treatment. It relies on the identification of factors associated with the risk of disease relapse after remission has been achieved and with the treatment response, and the further use of those factors to adapt to the intensity of treatment. Nowadays, large experimental analyses of proteins such as mass spectrometry and proteomics techniques permit a more precise identification of proteins associated with a disease. This work represents a useful review about analysis of protein samples from pediatric patients diagnosed with acute lymphoblastic leukemia (ALL), through mass spectrometry-based proteomic methodologies.

KEYWORDS: proteomics, mass spectrometry, childhood, leukemia.

ABBREVIATIONS

ALL	:	Acute Lymphoblastic Leukemia
HR	:	High risk
NCI	:	National Cancer Institute
EFS	:	Event-free survival
OS	:	Overall survival
CNS	:	Central nervous system
WBC	:	White blood cells
DNA	:	Deoxyribonucleic Acid
BM	:	Bone marrow
MS	:	Mass spectrometry
SCT	:	Stem cell transplant
LMIC	:	Low-middle income countries
PGR	:	Prednisone good response
PPR	:	Prednisone poor response
MRD	:	Minimal residual disease
PTM	:	Post-Translational Modifications

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1. Introduction

This review summarizes the studies that have analyzed protein samples acquired from pediatric patients diagnosed with acute lymphoblastic leukemia (ALL), through mass spectrometry (MS)-based proteomic methods. A PubMed® (https://pubmed.ncbi.nlm.nih.gov/. Last search September 2020) search was carried out with the following keywords: ((pediatric or children or childhood) AND (acute lymphoblastic leukemia or ALL)) AND (proteomic or proteomics)). All results were then cribbed to select only those original studies carried out on pediatric (less than 21 years) patient samples (serum, bone marrow,

cerebrospinal fluid) with ALL of any type (B or T lineage). It is important to note that only the proteomic studies achieved through MS were selected for this work.

This review consists of 3 sections: an overview of pediatric ALL; an overview of proteomics and MS-proteomics; and the description of published research combining these two fields of science aimed at the diagnosis, biology and treatment response of the disease.

2. Acute lymphoblastic leukemia

Pediatric cancers are a group of diseases of genetic and epigenetic origin that represent the first cause of death in the pediatric population in developed countries [1]. In Low-Middle income countries (LMIC) that have controlled deaths caused by infections and malnutrition, pediatric cancer also represents the first cause of death in this age group [2]. Of all the types of malignant tumors in children, there are 3 which have remained the most frequent causes of cancer for decades: acute leukemias, central nervous system (CNS) tumors, and lymphomas [3]. Almost all pediatric leukemias originate from immature hematopoietic precursors (acute leukemias), a different condition compared to adults, as they predominantly originate from mature hematologic cells (chronic leukemias) [4]. Globally, the incidence of acute leukemias represents 30% of all malignancies in children, but they can reach as much as 50% in certain populations, especially in Hispanic descendants [2, 3]. There are two types of acute leukemias, lymphoblastic and myeloid, and the difference depends on the lineage of the hematopoietic precursor from where the neoplasm arises [5].

ALL is the most common malignancy of childhood, representing almost a quarter of all cancers diagnosed in this age group, with an incidence of approximately 41 cases per million in kids from 0 to 14 years and 17 cases per million in the 15 to 19 year old age group in the United States [6]. Before 1948, ALL was considered a disease that rarely benefited from treatment of any sort, leading to the death of the patient in all cases. That year Sydney Farber reported a clinical trial in which major clinical, hematological, and pathological improvements were obtained with the use of an antimetabolite (aminopterin, which later led to methotrexate), although just for a few months [5]. As novel chemotherapeutic agents were being discovered (prednisone, vincristine, mercaptopurine and cyclophosphamide) to have antileukemic effects, their combined use became more common, which led Faber in 1966 to report the results of treating 1445 pediatric patients with 1% (15 patients) surviving, showing no evidence of disease for 5 years or longer. In that article the author quoted: “it is still impossible to differentiate, at the time of diagnosis, the 99% whose lives will be prolonged by months or one or two years and the 1% who will survive 5 years or longer” [7]. Little did he know that this observation would later constitute the main analysis strategy for further clinical trials, and which over time, have improved the 5 year overall survival to 90% [8].

The evolution of ALL treatment has been characterized by the identification of groups of patients that share one or more conditions that are related to the frequency of events of relapse once remission of the disease is achieved. This stratification method separates patients that have low probability of relapse (low-risk), based on previous studies, from those with a high probability (high-risk) [9]. Modern chemotherapeutic schemes adapt the intensity of treatment according to the risk of relapse, with the most intensive and toxic treatment reserved for high-risk (HR) patients [10].

As time passed, factors of prognostic significance were identified and later confirmed or discarded. These factors can be divided into three categories: those related to the patient, those related to the disease and those related with the response to initial treatment. The National Cancer Institute (NCI) criteria divide patients into standard and high-risk (HR) categories. Children between 1 and 9 years with a white blood cell (WBC) count at diagnosis of $<50 \times 10^9$/L fall into the standard risk category. Children less than 1 year or older than 10 years or any age with an initial WBC count of $>50 \times 10^9$/L are categorized as HR [11]. Disease factors that influence outcomes involve certain genetic and molecular alterations in leukemic lymphoblasts (rearrangements of KMT2A); hypodiploidy (<44 chromosomes or DNA index

<0.8); t(17;19)(q21-q22;p13.3) or the resultant fusion transcript E2A-HLF; intrachromosomal amplification of chromosome 21 (iAMP21); the fusion transcript BCR-ABL1 or t(9;22)(q34;q11); Ph-Like phenotype) [12-15]. Similarly, the lineage of the lymphoblast (B or T) affects the outcome, and hence T immunophenotype requires an intensification of the regime and greater use of cyclophosphamide and cytarabine [16, 17]. The presence of central nervous system (CNS) or testicular disease at presentation also puts a child at a higher risk of relapse [12] and a poor prognosis [18].

Treatment response is the most powerful predictor of outcomes. It is measured as the change in tumor burden at different timepoints and after different blocks of treatment. Some of these include: 1) Prednisone response after 7 days of treatment before the induction scheme (good-response (PGR) <1000 blast in peripheral blood; poor-response (PPR) otherwise); 2) Morphological analysis of the bone marrow; and 3) Minimal residual disease (MRD). MRD can be measured at several time points depending on a particular treatment protocol; the source can be peripheral blood or bone marrow and it can be analyzed with different technologies such as flow cytometry directed to the diagnostic immunophenotype of the clone, reverse transcription polymerase chain reaction (RT-PCR) searching a specific fusion gene transcript and allele-specific oligonucleotides polymerase chain reaction (ASO-PCR) directed to identify the specific rearrangement of the T-cell receptor (TCR) or immunoglobulin of the malignant cells [16-20]. Patients with slow or poor response to treatment, relapse earlier and more frequently [18, 21].

Finally, ALL is a disease benefited from personalized medicine. The best example of this is the discovery of a small inhibitor (imatinib) of the fusion protein BCR-ABL, which results from the presence of the Philadelphia chromosome (t(9;22)(q34;q11)). This protein causes an upregulation of the cell cycle and the inhibition of apoptosis, which produces a strong resistance to chemotherapy and poor outcomes. The use of imatinib dramatically improved the survival of this group of patients whose only option to achieve 50% of survival was through a Hematopoietic stem cell transplantation [22].

3. Proteomics

The term proteomics was coined by Marc Wilkins in 1994 to describe the study of the proteome. Proteome refers to the totality of proteins produced by a genome and its main objective is to understand how proteins work together to perform specific functions [23]. Over the years, proteomics has grown exponentially, going from the isolation of a small number of proteins and their sequencing, to the study of their three-dimensional structure, networks of interactions and post-translational modifications (glycosylation, phosphorylation, etc.), since they are the key knowledge of cell physiology [24]. Similarly, given that the presence of a protein under conditions in which it should not be present alters the function of the cell, proteins are ideal predictors of the biological behavior of cancer (aggressiveness, response or resistance to treatment, etc.) [25], and since many of the Hallmarks of cancer are a direct consequence of the functional contribution of one or several proteins (such as the sustained stimulus to proliferate, the evasion of the immune response and apoptosis) their pharmacological inhibition has laid the foundations for precision medicine, which is being actively investigated in both adult and child cancer clinical settings with promising results [26]. Finally, the implementation of proteomics in the improvement of the medical care of cancer patients is justified in that the identification of genetic mutations (translocations, deletions, amplifications) and the RNA expression profile (transcriptome) is not necessarily associated with the presence/absence of proteins (and the respective functional changes caused) and this is evidenced in the clinic with the observation that patients with similar genetic alteration patterns have shown mixed or contradictory results [25]. Examples of the above are investigations in different human body fluids such as serum, urine, prostate fluid, or mammary secretion for the timely diagnosis of some types of tumors [27].

The study of the proteome was complex in the past compared to the study of the genome, since the latter is static while the proteome is dynamic (protein expression changes over time and at different conditions while genes do not). Also, the human genome has approximately 25,000 genes (DNA), which can produce approximately 100,000 transcripts (RNA) whose processing and

subsequent combination can produce more than a million proteins. Furthermore, post-translational modifications (PTM) increase the complexity of proteomes compared to their corresponding genomes. Despite the above, the development of new technologies in protein separation, chemistry and bioinformatics throughout this century has made the study of the proteome possible and an expanding area of research [28, 29].

There are two different approaches in proteomics. One is the discovery of a protein through its selective isolation, the characterization of its sequence and three-dimensional structure, and the study of its functions, interactions, regulation, and post-translational modifications; this approach is also known as Top-Bottom. On the other hand, the second method is the Bottom-Up approach, or the so-called 'shotgun', as an analogy to the genomic shotgun studies reported by Venter in 1998 [24]. In this last strategy, the aim is to identify all the proteins present in a sample and it is on this that this review focuses.

The techniques used in shotgun proteomics focus on the preparation of samples from massive and/or complex sources, the separation of peptides, their detection by a mass spectrometer and the analysis of the information obtained in order to identify the greater amount of proteins present. The various protocols used for the preparation of samples obtained from patients, which are aimed at maximizing protein extraction and limiting sample loss, have been extensively summarized elsewhere [25, 30].

Once the sample has been prepared and digested, peptide separation can be based on the use of 2D gels (sodium-duodecyl-polyacrylamide (SDS-PAGE), two-dimensional electrophoresis (2-DE) and two-dimensional differential electrophoresis (2D-DIGE)) or liquid chromatography. The latter is the most used at the moment, given the difficulty in the reproducibility of the use of gels in the separation of hydrophobic proteins and that rare proteins (less than a thousand copies) are undetectable with this methodology [24, 31].

Mass spectrometry requires a low-energy ionization source that transfers peptides from solid/liquid to gaseous states (matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI)). Once the peptides are charged and in a gas phase they enter a vacuum chamber, where an analyzer detects the most frequent precursor ions; then they are isolated and fragmented (commonly in chambers filled with an inert gas such as nitrogen) to later be identified through the analysis of the mass/charge relationship (m/z) of its fragments. Currently, four basic types of mass analyzers are used: time of flight (ToF), ion trap, quadrupole (single and triple or tandem), and Fourier transform ion cyclotron resonance (FTICR) analyzers. All four differ considerably in sensitivity, resolution, mass precision, and peptide ion fragmentation potential [32]. Computer algorithms help enormously in the colossal task of identifying peptides and ultimately the protein from which they were fragmented.

4. MS-proteomic studies on childhood acute leukemia samples

As previously described, the success of the treatment of pediatric ALL relies on the identification of factors associated with the risk of disease relapse and their further use to adapt to the intensity of treatment [9, 10]. Pediatric leukemia has benefited from molecular biology developments. Currently, different types of DNA and RNA analyses (gain or loss of material, point mutations, translocations, etc.) are used on a daily basis to allocate patients to the arm of the treatment known to give the best results in that situation. The identification of the membrane proteins present in the malignant lymphoblast and the phosphorylation of selected intracellular pathways (jak-stat, MAPK, MTOR, among others) through Flow-cytometry are examples of how the presence of proteins in the tumor cells can help determine the medical treatment of leukemia patients. At the omics level, genomic and transcriptomic studies carried out in pediatric ALL are much more abundant than those of Proteomics.

This work covers about 15 original reports which are focused on the scope of our study. The efforts in the field of proteomics and ALL have aimed at: understanding the *biology* of the disease, identifying proteins or signature proteomes (or fingerprint protein expression patterns) associated with *treatment response*, and searching for protein

biomarkers for further validation for *diagnosis*. Table 1 summarizes the type of sample, techniques etc.

4.1. Biology

Lymphoid and myeloid cells differentiate in gene expression, in order to achieve their specific functions through protein production. During the hematopoietic process, gene expression constantly changes and hence the expression pattern of leukemia blasts depends on the linage of the precursor and the moment of maturation blockage. Hegedus C. M. *et al*. explored this situation and found evident differences in the expression pattern between lymphoid and myeloid as well as between subtypes of lymphoblastic leukemia. A truncated form of ubiquitin (absence of the three last amino acids at C-terminal) was found in lymphoblastic leukemia. This region of the protein mediates the union to other target proteins to degrade them through the ubiquitin-proteasome complex, concluding that cellular processes that regulate functions such as cell cycle, growth, proliferation, and apoptosis could be affected [33].

Changes in the level of expression of certain proteins may explain the biologic behavior of this disease; examples found through MS-proteomic methodologies are GSTP that neutralize intracellular toxic agents used during treatment and that may explain chemoresistance; or PHB that control cellular differentiation and morphogenesis, blocking maturation in a gene pattern that maintain a state of proliferation and apoptosis evasion. Up-regulation of HnRNPA2 may contribute to leukemogenesis as an oncogene which promotes proliferation [34]. Down-regulation has also been observed (PRDX4, 60s acidic ribosomal protein P0, actin) and it may promote the loss of control of processes like proliferation, differentiation, and tumor suppression [35].

Biologic changes according to genetic lesions were described. Costa *et al*. studied patients with t(12;21), which is the most frequent recurrent genetic lesion on pediatric patients and is associated with a good prognosis; this could be explained with the upregulation of CNN2, a cell proliferation inhibitor; PITPbeta, which decreases proliferation and increases apoptosis; the under expression of hnRNPE2 which would facilitate apoptosis; and CK2alpha whose absence prevent degradation of the tumor suppressor Ikaros *via* the ubiquitin pathway [34].

Tyrosine kinase receptors play a fundamental role in ALL as they transmit signals for proliferation after their stimulation. A research group used phosphoproteomics to interrogate 3 xenograft models derived from patients with ALL after stimulation of FLT3 and PDGF, and found that PAK1 and PAK2 were importantly regulated; also, the phosphorylation of their residues was decreased after treatment with FLT3 inhibitors (midostaurin and lestaurinib), which could base further use of these drugs in the context of precision medicine [36].

A proteomic study of cerebrospinal fluid (CSF) from ALL patients with CNS involvement found up-regulated proteins that could explain the process of infiltration. Proteins associated with the development of metastasis are: TIMP1 that inhibits the proteolytic activity of matrix metalloproteinases in the extracellular space, LGALS3BP that modulates cell-cell and cell-matrix interactions and FN1 that promotes cell adhesion and migration. They also reported down regulation of proteins of the complement system (C2 and C4a) that could explain the immunological evasion and survival of lymphoblast in the CNS after they accomplished the infiltration [37].

To gain insight into the transcriptomic repercussion of high hyperdiploid ALL and ETV6-RUNX1 positive cases, a proteogenomic study analyzed DNA, RNA and proteins isolated from bone marrow of these two types of pediatric ALL.

The analysis of copy number and gene/protein expression confirmed that the extra chromosomes in high hyperdiploid ALL have a large impact at RNA and protein levels in both cis and trans manner. When cases of hyperdiploid leukemia were compared with ETV6-RUNX1 positive cases, 1286 proteins were upregulated and 1127 were downregulated in the high hyperdiploid group [38].

4.2. Treatment response

By comparing ALL patients according to their prednisone response, a number of differentially

Table 1. Proteomic studies on ALL pediatric samples.

Author	Year	Country	Conditions studied	Source of sample	Objective	Proteomic strategy	Conclusions
Hegedus C. M. [33]	2005	USA	4 cell lines and 20 patients (5 ALL t(12;21), 6 ALL hyperdiploidy, 2 ALL and 5 AML normal karyotype, 1 AML inv(16) and 1 AML complex karyotype)	Bone Marrow and cell lines	To find differences between ALL and AML	Protein Chip Arrays SELDI-TOF	40 different peaks between ALL and AML. ALL express a truncated form of ubiquitin (3 last aa missed)
Lauten M. [39]	2006	Germany	4 PPR vs 8 PGR and 10 PPR vs 20 PGR	Bone marrow	To identify differences between patients with prednisone good and bad response	2-DE SELDI-TOF	5 peaks differentially expressed in PPR, 4 identified as Catalase, RING finger protein 22 alpha, VCP and GPRC5D. 5 peaks differentially expressed in PGR, 2 identified as protein kinase C beta 1 and Malate dehydrogenase 1
Verrils N. M. [41]	2006	Australia	1 VCR sensitive vs 1 intrinsically VCR resistant 1 VCR sensitive vs 1 acquired VCR resistant	Xenograft samples derived from pediatric ALL patients	Differences in protein expression dependent on vincristine sensitivity	2D DIGE SDS-PAGE MALDI-TOF	Intrinsic resistance to vincristine: Increased: gelsolin, HSC71, hnRNP H, TCP-1 beta, TCP-1epsilon.

Table 1 continued..

Author	Year	Country	Conditions studied	Source of sample	Objective	Proteomic strategy	Conclusions
							Decreased expression: moesin, class II beta-tubulin, Gamma actin Acquired resistance to vincristine: Increased: gelsolin, leukocyte elastase inhibitor protein, ezrin, hnRNP H Decreased: stathmin
Shi L. [45]	2009	China	94 ALL and 30 AML vs 54 healthy controls	Serum	To identify expression patterns and proteins to be used as diagnostic biomarkers for ALL	Protein chip arrays SELDI-TOF nano-LC-ESI-MS/MS	Five protein peaks with the highest discriminatory power were used in a classification tree with 91.8% sensitivity, specificity of 90% and positive predictive value of 90% to identify ALL cases
Jiang N. [40]	2011	Singapore	4 cell lines (1 GC resistant) Two samples (before and after GC treatment) of 43 ALL (35 PGR and 8 PPR)	Bone marrow	To identify possible biomarkers of prednisone response in ALL cell lines and validate in bone marrow of pediatric ALL patients	All cell lines 2-DE and MALDI-TOF Bone marrow Western blot	In patients with PGR there was down regulation of PCNA after GC treatment, in PPR PCNA remained unchanged after treatment

Table 1 continued..

Author	Year	Country	Conditions studied	Source of sample	Objective	Proteomic strategy	Conclusions
Braoudaki M. [42]	2013	Greece	45 ALL patients (19 LR and 26 HR)	Bone marrow cells and plasma Peripheral blood cells and plasma	Search for differences in protein expression on samples of patients with low risk and high risk ALL	2-DE MALDI-TOF	BM, plasma: Increased in HR: CERU, CLUS, THRB, AMBP, VTDB, FLN3 Decreased in HR: GELS, S10A9 Increased in LR: VTDB, KNG1 Decreased in LR: S10A9, AFAN PB plasma: Increased in HR: AMBP Decreased in HR: GELS Increased in LR: KNG1 Decreased in HR: AFAM
Wang D. [35]	2013	China	15 ALL vs 15 healthy controls	Bone marrow	Compare differential proteins between ALL and healthy controls searching for mechanisms that cause the disease and novel diagnostic and therapeutic strategies	SDS-PAGE MALDI-TOF	15 protein peaks, 8 identified Up regulated in ALL: GSTP, PHB Down regulated in ALL: PRDX4, 60s acidic ribosomal protein P0, actin, TPI-1, PNPO, FLJ26567

Table 1 continued..

Author	Year	Country	Conditions studied	Source of sample	Objective	Proteomic strategy	Conclusions
Costa O. [34]	2014	France	13 ALL (6 with t(12;21) vs 7 with other genetic lesions)	Bone marrow	Analyze protein expression on ALL with an specific genomic lesion (t(12;21) ETV6-RUNX1)	2-DE nanoLC Ion trap MS/MS	Proteins differentially expressed in the t(12;21) group Up regulated: CNN2, MAT2beta, HnRNPA2 Down regulated: hnRNP-E1,BUB3alpha, SEPT9_i4, CK2alpha
Priola G. M. [44]	2015	USA	5 ALL patients (1 positive for CNS thrombosis, 4 not) measured on day 0, 8 and 29 of induction therapy	Cerebrospinal fluid	To evaluate the feasibility of proteomic studies on CSF to find differences in patients who develop CNS thrombosis	LC-MS/MS	Patient with CNS thrombosis: decreased SERPINA5, SERPINC1, plasminogen, Factor X
Cavalcante M. S. [46]	2016	Brazil	10 ALL (sampled at diagnosis and at the end of induction therapy) vs 10 healthy controls	Serum	To identify early diagnosis biomarkers of ALL to further validate	FPLC nanoUPLC-ESI-MS/MS	Over-expressed in ALL but not in control or post induction therapy: LRG1, CLU, F2, SERPIND1, A2M, SERPINF2, SERPINA1, CFB and C3

Table 1 continued..

Author	Year	Country	Conditions studied	Source of sample	Objective	Proteomic strategy	Conclusions
Xu G. [43]	2017	China	12 ALL (6 low-medium risk vs 6 high risk) And 6 controls	Bone marrow	To find critical altered proteins in high risk ALL	LC-ESI-MS/MS	54 proteins with higher expression in HR ALL than LR, and that were not found in control samples.
Siekmann I. K. [36]	2018	Germany Canada, UK	3 ALL patients	Xenograft s models derived from patients	To decipher signaling circuits that link RTK activity with biological output in vivo, with dependencies on FLT3 and PDGFRB	Phosphoproteomics iTRAQ nano-LC MS/MS	A total of 7319 phosphosites corresponding to 2173 unambiguous phosphoproteins were identified. PAK1 and PAK2 most significant ones.
Calderon-Rodríguez S. I. [47]	2019	Colombia	6 B-ALL vs 6 healthy controls	Plasma	To examine plasma from B-ALL patients searching for diagnostic markers	nano-LC MS/MS	25 proteins identified in B-ALL FYV1, PGCA, CSF1R, F13A, CELR2, CRIS3, FBLN1, SHBG, NCHL1, FIBG, TRIM1, CAD13, FIBB, GELS, CHLE, F13B, FIBA, LUM, CETP, LV403, HRG, MEGF8, FETUA, PI16 and MASP2

Table 1 continued..

Author	Year	Country	Conditions studied	Source of sample	Objective	Proteomic strategy	Conclusions
Guo L. [37]	2019	China	6 ALL with CNS involvement vs 6 controls	Cerebrospinal fluid	To identify protein biomarkers expressed in newly diagnosed ALL	LC-MS/MS	51 proteins found (32 up regulated and 19 down regulated)
Yang M. [38]	2019	Sweden, Germany, and UK	18 high hyperdiploid and 9 ETV6-RUNX1	Bone marrow	A proteogenomic study to compare transcriptional differences between high hyperdiploid and ETV6-RUNX1 ALL cases	HiRIEF LC-MS/MS	In hyperdiploid cases: 1286 proteins were up regulated (importantly CD44 and FLT3) and 1127 down regulated (IGF2BP1, CLIC5, RAG1 and RAG2).

ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, PGR: prednisone good response, PPR: prednisone bad response, VCR: vincristine, GC: glucocorticoid LR: low risk, HR: high risk, CNS: central nervous system.

expressed proteins have been reported that are linked to cellular processes like cell growth (RING finger protein and protein kinase C), related to hematopoiesis and immunity (G-protein coupled receptors), apoptosis (VCP) and cell division (Malate dehydrogenase). The presence of VCP in patients with poor prednisone response (>1000 blast cells in peripheral blood 8 days after the start of prednisone) could explain a decrease in the apoptosis pathway of leukemic blasts [39]. Others found that when measuring PCNA at the beginning (day 0) and the end (day 8) of prednisone treatment, if the level of expression of this protein remained unchanged, it correlated with the bad outcome of prednisone poor responders [40].

Resistance to vincristine can be developed if there is a change in cytoskeletal composition (decrease class II beta tubulin), if there is a decrease in microtubule destabilizers (stathmin) or an increased production of stabilizers (HSC70, TCP-1). The proteomic evidence for the development of Vincristine resistance are cytoskeletal changes principally of proteins associated with actin (gamma actin) and tubulin (class ii beta tubulin), the involvement of proteins that coordinate signaling and structural links between plasma membrane and cytoskeleton (Ezrin, moesin and CapG), blockage of apoptosis (gelsolin) and a reduction of microtubule destabilizers (stathmin) that may counteract the effects of the drug [41].

Biomarkers to identify High and Low risk of relapse patients have been proposed, among them are GELS, S10A9, AMBP, ACTB, CATA, AFAM, KNG1 but further evaluation is needed [42]. Also, Xu *et al.* performed a shotgun study in bone marrow from 12 ALL patients (6 high risk and 6 low risk) and 12 controls and identified 86 proteins that were highly expressed in HR patients. From these, 35 show direct protein-protein interaction around pathways of cell growth and development (Hsp90beta), DNA splicing and damage response (YBX1, DDX48). They further used this information to evaluate the level of expression by western blot in a new set of BM samples (24 L/MR and 18 HR) and found the same expression profile, with the addition of hsp90 alpha and Thrp3, which are known as part of the same pathways. These findings could explain the biologic behavior of HR lymphoblasts as they have altered processes like DNA damage and stress response, as well as RNA splicing that could further disrupt other key pathways like cell cycle control and apoptosis. Also, with this data, hsp90 inhibitors represent new candidate drugs for HR ALL patients [43].

During chemotherapy administration, one of the strongest obstacles to face is the presence of treatment complications. By comparing the evolution of protein expression in CSF of children with ALL on days 0, 8 and 29 of induction therapy with or without the presence of CNS thrombosis, it has been proposed that basal deficiencies in the coagulation pathway in combination with acquired deficiencies during asparaginase treatment could be the cause of this complication. Also, the authors propose to validate the search of these basal deficiencies as predictors for CNS thrombosis [44].

4.3. Diagnosis

Patients with acute lymphoblastic leukemia seek medical attention when non-specific signs and symptoms appear, which makes it difficult for first contact physicians to add it to the list of differential diagnoses. Thus, the development of a highly sensitive, specific, and low-cost set of biomarkers that allow ruling out the diagnosis in the clinical setting, when ALL is suspected, is justified. In this regard, Shin L. *et al.*, proposed in 2009, a classification algorithm based on 5 proteins' peaks (4 identified as CTAP-III, PF4 and 2 fragments of C3a) found by proteomic studies that could identify ALL cases with 91.8% sensitivity, 90% specificity and positive predictive value of 90% [45]. Also Cavalcante M. S. *et al.* found 9 proteins (LRG1, CLU, F2, SERPIND1, A2M, SERPINF2, SERPINA1, CFB and C3) over expressed in serum of ALL patients before the beginning of treatment that were significantly different to control patients and that disappeared from plasma of the same patients after they achieved remission of the disease, suggesting that they were attractive biomarkers to further evaluation [46]. Finally, Calderón-Rodríguez S. I. *et al*. proposed 12 proteins (CSF1R, CAD13, FBLN1, PGCA, SHBG, NCHL1, TRIM1, CELR2, CRIS3, F13A, FIBB and FIBG) that were found

downregulated in Colombian pediatric ALL patients when compared to healthy controls [47].

It is fundamental to assess CNS involvement at diagnosis to define whether or not to add intrathecal chemotherapy or radiotherapy. A Chinese group compared the protein expression of 6 samples of CSF from CNS-involved ALL pediatric patients with 6 pediatric controls, and found 51 differentially expressed proteins (32 up regulated and 19 down regulated) that could be used as biomarkers as their level of expression was statistically significant [37].

5. Conclusions

The study of the protein expression patterns directly on malignant lymphoblasts, although limited now, will add a new dimension in the diagnosis, risk of relapse stratification and follow up of pediatric patients with acute lymphoblastic leukemia. Also, it will improve, coupled with analysis of DNA and RNA, the strategy of precision medicine, which aims at the identification of drug targets, genomic alterations and expression patterns that are known to respond to specific drugs with mechanisms of action different from those of conventional chemotherapy.

CONFLICT OF INTEREST STATEMENT

The authors declare that no competing interest exists.

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