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REVIEW ARTICLE

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Metabolic profile analysis in hematological malignancies

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Abstract

Metabolomics, an emerging discipline, analyzes the totality of metabolites in cells, tissues, and fluids, providing real-time insights into the metabolic state of an organism. Recent advances in technology and databases have driven research focused primarily on the pathophysiology of solid tumors. However, metabolomics is also gaining importance in hematopoietic neoplasms, particularly acute myeloid leukemia and multiple myeloma. This review discusses potential novel biomarkers for diagnosis, risk assessment, and treatment response in hematological cancers that offer promising prospects for personalized therapies.

Keywords: metabolomics, metabolism, hematology, biomarkers, hematopoietic neoplasm

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Introduction

Reading the entire genetic code sequence has prompted further research into the relationship between gene expression and pathophysiological processes occurring in the body. This led to the development of other fields of systeomics, such as transcriptomics in which mRNA is analyzed, and proteomics, which focuses on the study of proteins [1]. Changes in the genome, transcriptome, and proteome may indicate a predisposition to specific biological processes, but they do not determine the current physiological state of the organism [2].

Metabolomics, defined as qualitative and quantitative analysis of the metabolome — the complete set of metabolites present in cells, biological fluids, and tissues — is one of the newest fields of systeomics [3]. By capturing the state of metabolism in a biological system at any point in time, metabolomic analysis is a valuable tool in characterizing the pathophysiological state of an organism [4, 5]. In recent years, significant technological, methodological, and computational advances have led to the maturation of this field [6]. Creating specific databases containing accurate information on metabolites found in humans appears to be particularly helpful [7–9].

All this has spearheaded rapid progress in research, with an increasing number of papers describing changes in the metabolomic profile in cancer patients. Most studies have related to the pathophysiology of carcinogenesis, mainly concerning solid tumors, particularly in breast and lung cancers [10–14]. Recently, there has been greater interest among researchers in the field of hematopoietic and lymphoid cell neoplasms. There have been an increasing number of studies carried out assessing changes in metabolite concentrations, particularly in acute myeloid leukemia (AML) [15–17] and multiple myeloma (MM) [18–20]. Despite the significant development of diagnostic and therapeutic methods seen in recent years, some hematological diseases remain incurable. Therefore, identifying new biomarkers, especially those indicating treatment response, and potential new targets for precision drugs, appears to be increasingly essential.

This paper reviews current metabolomics research in blood cancers concerning the therapeutic process: diagnosis, risk stratification of disease failure, and assessment

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of treatment response. The final section describes the challenges underlying metabolomics-based experiments and discusses the potential pitfalls of the analytical process, including study design and sample collection (Table I [18, 19, 21–28]).

Metabolomics as a diagnostic biomarker

Recent years have seen substantial improvements in the diagnosis of hematological neoplasms, primarily due to advances in cytogenetics, molecular biology, and flow cytometry [29], which in turn have led to changes in diagnostic criteria. The latest 2022 World Health Organization (WHO) classification has updated and modified the long-standing paradigm of a requirement for at least 20% of blast cells necessary for diagnosing AML. With the coexistence of specific unfavorable molecular abnormalities, this has been reduced to 10% [30].

However, bone marrow biopsies, which are still the diagnostic 'gold standard' in most hematological diseases, are invasive procedures associated with possible complications for the patient. In addition, making a histopathological diagnosis depends on the experience of the pathomorphologist. This has resulted in a search for new, and often less invasive, biomarkers to make the diagnosis [31].

Musharraf et al. [21] carried out serum metabolite analysis of 186 patients with AML, acute lymphoblastic leukemia (ALL), or aplastic anemia (APA) with the serum of healthy subjects using high-resolution magnetic resonance spectrometry (MRS) and gas chromatography-mass spectrometry (GC-MS). Concentrations of glycolysis metabolites, the citric acid cycle, lipoproteins, and fatty acids (FAs) varied between the serum of AML, ALL patients, and healthy controls. Significant differences were found in the concentrations of FAs — especially palmitic, stearic, and oleic acids. Palmitic acid concentrations were reduced in AML and ALL patients, while oleic and stearic acid concentrations were significantly elevated, compared to healthy controls. FAs serve as the fundamental components of lipids and play a vital role in forming the structural basis of biological membranes. Moreover, FAs have other functions, such as acting as storage or signaling molecules, and additionally they can be used as a source of energy through oxidation to produce carbon dioxide (CO_2). Acute leukemia is a proliferation in which many immature cells continuously proliferate in the bone marrow, resulting in a high metabolic demand [32]. The changes in FA concentrations may be due to their oxidation and use by blast cells to generate energy, enabling survival under metabolic stress. Interestingly, serum carbohydrate levels have been shown to be significantly reduced in all serum samples from diseased individuals compared to serum levels in healthy controls. Carbohydrates are commonly used in the metabolism of FA synthesis [33]. It has been suggested that the significant reduction in serum hydrocarbon levels in cancer patients may be related to increased metabolism of FA synthesis. Assessment of lipid metabolite concentrations can be helpful in the earlier identification of diagnostic biomarkers of cancer cell acute proliferation.

Petrick et al. [22] analyzed the metabolomic profile of 48 pediatric patients with AML and 46 healthy subjects in archived dry blood samples (DBS) routinely collected up to 48 hours after birth to look for possible markers indicating an increased likelihood of AML. With male sex a known risk factor for developing AML in children, patients were divided into groups based on sex. They showed that changes in metabolites measured at birth could differentiate neonates later diagnosed with AML. The analyzed sphingolipid metabolism in girls who developed acute leukemias showed differences in ceramide concentrations compared to healthy individuals.

Ceramides are integral components of the sphingolipid metabolism, which plays a central role in signaling pathways and has various implications for cancer cell proliferation and death [34]. Since the levels of sphingolipid molecules are tightly controlled by metabolic enzymes, any changes in their expression or activity can have significant effects on the induction of cancer cell death or survival [35]. Similar abnormalities in sphingolipid metabolism have been found in other hematological neoplasms as well as in breast cancer and other acute leukemias [36–38]. These findings suggest a potential disruption in sphingolipid metabolism, possibly involving dihydroceramide synthases, which play unique roles in regulating cell death in cancer. Moreover, the altered sphingomyelin profiles may influence cell membrane fluidity, which is crucial for signal transduction and ion transport.

In addition, in the female study group, two metabolites associated with the risk of developing AML showed a strong correlation with breastfeeding duration, which may indicate breastfeeding to be a protective factor against the disease. Interestingly, changes in metabolite concentrations in male infants who developed AML were more variable and showed no association with known risk factors. This suggests a gender difference in etiology, and indicates that the pathophysiology of acute leukemia development in males may be more multifactorial [39, 40].

Metabolomics as a biomarker of prognosis

Currently, scoring systems are known in most hematooncological diseases, such as Revised Multiple Myeloma International Staging System (R-ISS) or Revised International Prognostic Index (R-IPI) in diffuse large B-cell lymphoma (DLBCL). These scales, in addition to remaining reliable, validated tools for predicting patient outcomes, might also be helpful in individualized, risk-adapted initial treatment decisions and predicting outcomes for appropriate

Table I. Summary of selected metabolomic studies (compiled from [18, 19, 21–28])

Author [ref.]	Study	Sample	Diseases	Major technique	Major Findings	Pathways
Musharraf et al. [21]	Serum metabolomics of acute lymphoblastic leukaemia and AML for probing biomarker molecules	Serum of 186 adult patients	ALL, AML, APA	GC-MS	↑ oleic acid ↑ stearic acid ↓ palmitic acid	Fatty acid metabolism
Petrick et al. [22]	Untargeted metabolomics of newborn dried blood spots reveals sex-specific associations with pediatric AML	DBS of 94 pediatric patients	AML	LC-MS	↑ $C_{37}H_{75}N_2O_6P$ ↑ $C_{40}H_{79}NO_3$ ↑ lactosylceramide	Sphingolipids metabolism
Gonsalves et al. [18]	Metabolomic and lipidomic profiling of bone marrow plasma differentiates patients with monoclonal gammopathy of undetermined significance from MM	Bone marrow plasma of 50 patients	MM, MGUS	LC-MS	↑ 3-hydroxy-kynurenine ↓ valine ↓ leucine ↓ phosphatidylethanolamine ↓ lactosylceramide	Branched-chain amino acids metabolism Tryptophan metabolism Phospholipids metabolism
Veskovski et al. [19]	Serum metabolomic profiling correlated with ISS and clinical outcome for MM patients treated with high dose melphalan and autologous stem cell transplantation	Bone marrow plasma and serum of 202 patients	MM	^{1}H-NMR	In ISS I patients compared to ISS III patients ↓ valine ↓ leucine	Amino acid metabolism
Schraw et al. [23]	Metabolomic profiling identifies pathways associated with minimal residual disease in childhood acute lymphoblastic leukemia	Bone marrow plasma of 155 pediatric patients	ALL	LC-MS	↑ malate ↑ fumarate	Glycolysis Citric acid cycle
Reikvam et al. [24]	Pretransplant systemic metabolic profiles in allogeneic hematopoietic stem cell transplant recipients — identification of patient subsets with increased transplant-related mortality	Serum of 92 patients	Allo-HSCT complications	LC-MS	↑ lysine ↑ adenosine ↓ glycine	
Fei et al. [25]	Plasma metabolites forecast occurrence and prognosis for patients with diffuse large B-cell lymphoma	Serum of 65 patients	DLBCL	GC-MS	↑ malate	Citric acid cycle metabolism, branched-chain amino acids
de Almeida et al. [26]	Bioactive lipids as chronic myelogenous leukemia's potential biomarkers for disease progression and response to tyrosine kinase inhibitors	Serum of 38 patients	CML	LC-MS	↑ ceramide ↓ sphingomyelin	Lipid metabolism
Stockard et al. [27]	Cellular metabolomics profiles associated with drug chemosensitivity in AML	Seven AML cell lines	AML	UHPLC-MS	Doxorubicin refractory: ↑ glutamine ↑ glycine ↑ serine ↓ lysoPC 16:1 ↑ glycerol	Amino acid metabolism Lipid metabolism

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Table I (cont.). Summary of selected metabolomic studies (compiled from [18, 19, 21–28])

Author [ref.]	Study	Sample	Diseases	Major technique	Major Findings	Pathways
Puchades-Carrasco et al. [20]	MM patients have a specific serum metabolomic profile that changes after achieving complete remission	Serum of 31 patients	MM	^{1}H-NMR	↑ lactate ↑ lysine in remission	Amino acid metabolism
DiNardo et al. [28]	Serum 2-hydroxyglutarate levels predict isocitrate dehydrogenase mutations and clinical outcome in AML	Serum of 223 patients	AML	LC-MS	2-hydroxyglutarate as CR marker in IDH1(+) AML patients	Isocitrate dehydrogenase metabolism

ALL — acute lymphoblastic leukemia; allo-HSCT — allogeneic hematopoietic stem cell transplantation; AML — acute myeloid leukemia; APA — aplastic anemia; CR — complete remission; DLBCL — diffuse large B-cell lymphoma; GC-MS — gas chromatography-mass spectrometry; ^{1}H-NMR — proton (hydrogen-1) nuclear magnetic resonance; ISS — International Staging System; LC-MS — liquid chromatography-mass spectrometry; lysoPC — lysophosphatidylcholines; MGUS — monoclonal gammopathy of undetermined significance; MM — multiple myeloma; UHPLC-MS — ultra-high performance liquid-mass spectrometry

stratification and interpretation of clinical trials. Considering the heterogeneity of hematological diseases, and the emergence of new, and often targeted, therapies, the risk scales are frequently revised by the addition of new factors, especially cytogenetic or molecular ones. Changes in metabolite concentrations seem to be promising biomarkers of risk stratification.

Analysis of the bone marrow (BM) metabolite profile of 50 patients with MM and monoclonal gammopathy of undetermined significance (MGUS) showed significant differences in the concentrations of lipids and some amino acids [18]. Variations in the levels of branched-chain amino acids such as valine, leucine, and isoleucine, and their associated metabolites, distinguish patients with MGUS from those with MM. The main role of these amino acids in the body is the synthesis of proteins. They can also be metabolized to supply carbon compounds for different anabolic and metabolic processes such as gluconeogenesis and FA synthesis. Additionally, they can be a source of energy for the citric acid cycle [41]. The bone marrow of MM patients has been shown to be characterized by a significant reduction in branched-chain amino acids, suggesting their consumption for energy production by proliferating plasma cells. Furthermore, it has been observed that there were higher levels of tryptophan catabolism products, including increased quantities of 3-hydroxy-kynurenine. This increased concentration of 3-hydroxy-kynurenine is probably related to excessive activation of the indoleamine 2,3-dioxygenase 1 (IDO1) enzyme by plasma cells. Kynurenine metabolites may exhibit immunosuppressive effects leading to the inhibition of T-effector cell proliferation and increasing the production of T-regulatory cells, allowing clonal plasma cells to avoid the immune response in MM [42]. IDO1 has been shown to be overactive in several other human cancers [43]. Additionally, the study found reduced levels of phospholipids such as phosphatidylethanolamine (PE), lactosylceramide (LCER), and phosphatidylinositol (PI). MGUS is considered as a pre-cancerous condition with an annual risk of progression to MM of c.1% [44]. Given the need to search for further biomarkers of the more aggressive clinical course of plasma cell dyscrasia, metabolomic analysis would appear to be an exciting marker of disease progression.

For diagnosed MM, Veskovski et al. [19] attempted to assess serum and bone marrow plasma metabolite concentrations of 201 MM patients and compare these concentrations according to the MM International Staging System (ISS) risk groups in patients before a planned autologous stem cell transplantation (auto-SCT) procedure. It was shown that the levels of the two branched-chain amino acids leucine and valine were significantly lower in patients at ISS stage I than in ISS stages II/III, which is traditionally associated with a worse prognosis [45]. This is most likely related to plasma cells' significant consumption of these two amino acids. Furthermore, additional reports have highlighted changes in the inverse relationship of another amino acid, threonine, with the risk grade based on ISS and the number of plasma cells in the bone marrow. It appears that low levels of threonine may be a specific potential indicator for predicting the risk of developing MM.

In another study, an analysis of the bone marrow metabolomic profile of 155 pediatric ALL patients collected before the start of chemotherapy showed significant differences in the specific metabolite levels, depending on the patient's subsequent minimal residual disease (MRD) status, assessed at the end of induction treatment [23]. These differences were mostly related to carbon and amino acid metabolism. Samples of MRD-positive patients showed a decrease in glucose and an increase in levels of various metabolites related to glycolysis, the pentose-phosphate pathway, and the citric acid cycle. The introduction of metabolomic data to existing clinical risk factors such as immunophenotype or cytogenetic abnormalities resulted in an improved prediction of MRD. Subsequently, given the strong correlation between changes in carbohydrate metabolism and MRD, the focus has been on the role of nicotinamide phosphoribosyl transferase (NAMPT). NAMPT is an enzyme that plays a crucial part in generating oxidized

form of nicotinamide adenine dinucleotide (NAD+) through the salvage pathway. It facilitates the conversion of nicotinamide and 5-phosphoribosyl-1-pyrophosphate into nicotinamide mononucleotide (NMN), which is an important intermediate in the synthesis of NAD+. A key role for NAD+ in glycolysis is its ability to accept hydride equivalents, forming reduced form of NAD (NADH) during adenosine triphosphate (ATP) synthesis [46]. Other studies have shown that inhibition of NAMPT activity leads to a decrease in the flow of the TCA cycle, resulting in the depletion of malate and fumarate stores in cancer cells such as ovarian, colorectal, and MM cells [47–49]. Researchers have used various NAMPT inhibitors, including FK866, to assess clinical utility on ALL cell lines. They significantly reduced malate and fumarate metabolites strongly associated with MRD. Post-induction MRD assessment remains a crucial indicator of relapse risk in ALL [50]. To prevent relapse in patients with MRD-positive ALL, intensification of conventional chemotherapy is often used, which is associated with significant toxicity [51]. Assessing the risk probability of MRD-positive patients before starting induction treatment allows earlier identification of high-risk patients who may require other therapeutic strategies, such as those acting on carbon metabolism.

Despite the newer treatments being used, the only method of completely curing some hematological diseases is allogeneic bone marrow stem cell transplantation (allo-SCT) [52]. Findings indicate that metabolites are essential regulators of vascular, renal, endothelial, and gastrointestinal cell function, as well as the function of various immune system cells [53, 54]. A recently published study by Reikvam et al. [24] indicates the potential role of assessing the metabolite profile before a planned allo-SCT procedure. They compared the serum metabolite profile of patients before myeloablative conditioning according to three transplant-related mortality (TRM) complications: 1) acute graft-versus-host disease (aGvHD); 2) extensive fluid overload (FO); and 3) pretransplant inflammation (pTI). Allthese three complications led to specific changes in the pretransplant metabolic profiles, particularly affecting amino acid metabolism. However, it is important to note that there was a small overlap in the metabolites associated with aGvHD and inflammation or fluid retention. aGvHD was mainly related to disturbances in tryptophan, biotin, and phenylacetate metabolism and disruptions in the urea cycle regulation.

In contrast, pTI was mainly associated with disturbances in glycine metabolism. The reduction in glycine levels has a notable impact on different types of immune cells, including monocytes or macrophages, where it inhibits the release of cytokines mediated by toll-like receptor 4 (TLR-4). Additionally, glycine plays a protective role in kidney and endothelial cells. Excessive fluid retention was mainly associated with disturbances in adenosine metabolism. Adenosine serves as a controller for blood circulation and the permeability of small blood vessels. Moreover, T-cells express adenosine receptors, and adenosine plays a crucial role in regulating the initial phases of T-cell activation. Furthermore, Reikvam et al. [24] identified a group of metabolites most related to the occurrence of these complications. Given the high risk of death during and after bone marrow transplantation, metabolite profile analysis appears to be a promising new tool to facilitate clinical decision-making before the allo-SCT procedure.

Fei et al. [25] undertook a serum analysis of 65 patients DLBCL. They assessed the metabolite profile before introducing standard immunochemotherapy in relation to treatment response i.e. non-response (NR) and complete response. The analysis showed characteristic grouping, suggesting the potential for prognosis based on metabolite analysis. Patients in the CR group had similar concentrations of test compounds as the control group and significantly different concentrations from the NR group. These results demonstrate that specific abnormalities in the citric acid cycle metabolism, branched-chain amino acids (BCAAs), and methionine in patients with newly diagnosed DLBCL are closely associated with unfavorable prognoses. Furthermore, high malate concentrations have been identified as an unfavorable biomarker of treatment response [25]. There is increasing evidence that malate accumulation may be necessary for clonal proliferation of tumors and may contribute to increased glycolysis [55, 56]. Positron emission tomography–computed tomography (PET-CT) scanning provides valuable measurements of tumor metabolism and activity in DLBCL, and parameters such as maximum standardized concentration (SUV_{max}), metabolic tumor volume (MTV), and tumor lesion glycolysis (TLG) have not only proven their value in metabolic characterization and staging assessment, but also in evaluating responses to treatment [57]. Interestingly, malate and 2-hydroxy-2-methylbutyric acid levels were shown to be positively correlated with MTV and TLG.

Some hematological malignancies are characterized by a heterogeneous course [58]. In some patients, the disease has an aggressive approach and requires prompt initiation of treatment, while in others an indolent course does not require therapy for many years. In the case of chronic lymphocytic leukemia (CLL), Piszcz et al. [59] demonstrated in a group of 85 patients with this disease an increase in metabolites such as linoleamide or acyclocarnitine, whose serum levels were significantly increased in those with an aggressive course compared to those with an indolent course.

Metabolomics as an early biomarker of treatment resistance

Chronic myelogenous leukemia (CML) is a cancer that expresses the Philadelphia chromosome and the constitutively activated tyrosine kinase BCR-ABL in hematopoietic

progenitor cells. Despite the introduction of BCR-ABL inhibitors, some patients show resistance to treatment and have a worse clinical course [60]. The serum metabolic profile of patients responding and refractory to treatment with tyrosine kinase inhibitors has been assessed and compared. Lipid metabolism has been shown to differ significantly between these groups [26]. Notably higher ceramide and lower sphingomyelin levels were found in the serum of TKI-resistant patients compared to the treatment-resistant group. In addition, changes in the concentrations and metabolism of androgens, estrogens, and glycerophospholipids have been described. Since the only biomarker for treatment response in CML is still the BCR-ABL transcript, disturbances in lipid metabolism seem to be a promising therapeutic option. The studies described here reveal a significant role for metabolomics as a link to other fields of systeomics. Molecular perturbations and the use of an appropriate inhibitor alone are insufficient to eradicate a tumor clone. Only the study of the phenotype of the tumor cells with the help of metabolomics, which is not only genome-dependent, can determine the risk of treatment resistance at an early stage.

In AML, standard anthracycline- and cytarabine-based chemotherapy has been used for several decades. Despite this treatment, relapses are observed in c.50% of adult patients, in most cases leading to death [61]. Therefore, markers that predict resistance to standard chemotherapy have long been sought. To identify metabolic pathways that may play a role in the development of drug resistance, a metabolomic profile analysis was performed for seven AML cell lines with different sensitivities to standard chemotherapy [27]. Increased nucleoside levels were observed in cytarabine-resistant cells. Metabolic pathway analysis indicated significant changes in purine metabolism in the resistant cell lines. Elevated nucleoside levels may be related to the mechanism of cytarabine resistance through the activation of dephosphorylating enzymes, leading to the inactivation of the drug. Meaningful disturbances of amino acid metabolism were found in doxorubicin-resistant cells. Increased asparagine levels are a classic example of the altered amino acid metabolism observed in acute leukemia.

There is increasing evidence that AML cells have a similar asparagine dependence and susceptibility to asparaginase as ALL cells [62, 63]. The results suggest that the clinical relevance of asparaginase may be particularly essential in cases of doxorubicin resistance. In addition, increased levels of amino acids such as glutamine, glycine, and serine involved in synthesizing glutathione, which protects cancer cells from chemotherapy-induced oxidative stress, were found [64, 65]. Notably, doxorubicin-resistant cells showed changes in lipid concentrations — low concentrations of lysoPC 16:1 and high concentrations of glycerol were found. This indicates increased lipid and triglyceride catabolism activity, contributing to FA oxidation. Improving our understanding of this chemotherapy resistance may lead to the development of systems for selecting initial therapy, and open up new perspectives to improve AML treatment.

Metabolomics as a biomarker of treatment response

Increasingly sensitive imaging and laboratory methods are making it possible to assess treatment response with growing precision. One of the first applications of metabolomics recognized by researchers was to evaluate the effectiveness of a treatment.

As early as 2013, the results of a study by Puchades-Carrasco et al. [20] indicated that patients with MM who achieved complete remission showed differences in their serum metabolic profile compared to the time of diagnosis. Patients in remission had significantly higher levels of substances such as lactate, lysine, trimethylamine N-oxide (TMAO), choline, and cholesterol than did patients with active MM. In addition, the proposed statistical model indicated that once complete remission is achieved, the metabolic profile of MM patients approaches that of healthy individuals.

Mutations in the enzymes isocitrate dehydrogenase 1 and 2 (IDH1, IDH2) are found in c.30% of AML patients. These lead to loss of dehydrogenase function and the production of 2-hydroxyglutarate (2HG) instead of α-ketoglutaric acid (AKG). Elevated levels of 2HG in a patient's serum can be used as a diagnostic marker for IDH mutations and to monitor response to treatment. DiNardo et al. [66] demonstrated in a sample of 223 patients with newly diagnosed AML that diagnostic measurements of serum 2HG can allow rapid and accurate identification of AML patients with IDH mutations. In addition, it was shown that lowering serum 2HG levels below 200 ng/mL in patients in complete remission (CR) was associated with a statistically significant improvement of overall survival (OS).

This indicates that the 2HG assay could be a sensitive tool to assess residual leukemic cells after induction treatment, and might serve as a marker of MRD that could be used for therapeutic decision-making.

Therapeutic applications of metabolomics research

Targeting tumor metabolism is not a new treatment strategy: longstanding compounds such as methotrexate or nucleotide analogs e.g. pyrimidine (gemcitabine, cytarabine) or purine (fludarabine) are still widely used in clinical practice. Unfortunately, this is often associated with significant systemic toxicity. Thanks to new laboratory and bioinformatics technologies, deepening metabolomic research is identifying other handle points for new molecules, the use of which will be associated with less toxicity. Mutations in IDH1/2 provide a clear example of how observations of

metabolomic profiles and changes in metabolite levels in AML patients can be successfully applied to therapies for acute leukemia. Ongoing preclinical and clinical studies have led to the development of new treatment strategies, such as IDH1 and IDH2 mutant inhibitors. These drugs exert anti-tumor effects by inhibiting the production of 2-hydroxyglutaric acid. A phase I study of ivosidenib (an IDH1 inhibitor), conducted on 258 patients with relapsed/refractory AML with an IDH1 mutation, showed an overall response rate (ORR) of 41.6% and a CR rate of 21.6% in the primary analysis, leading to pre-Food and Drug Administration (pre-FDA) registration of the drug in the indication of refractory or relapsed AML (R/R AML) [28]. Studies of ivosidenib in combination with FLAG [fludarabine, cytarabine, (G-CSF) granulocyte colony-stimulating factor] chemotherapy for R/R AML and in first-line treatment in combination with a hypomethylating drug (azacytidine) have also been carried out (phase III AGILE study — NCT03173248) [67], leading to a pre-FDA registration of ivosidenib with azacytidine in May 2022 for the treatment of AML in patients ineligible for standard chemotherapy.

Studies are also under way to evaluate the efficacy of the IDH-1 inhibitor for maintenance treatment in patients undergoing allo-SCT and in other hematological malignancies such as myelodysplastic syndrome (MDS). The second IDH inhibitor registered in the R/R AML indication is enasidenib (IDH2 inhibitor). The registration trial (phase II/III study IDENTHIFY NCT02577406) showed an ORR of 40.5% and a median survival time (OS) of 6.5 months, leading to registration in the R/R AML indication [68].

Another example is the trial of therapy with the APO866 molecule. APO866 acts as an inhibitor of nicotinamide phosphoribosyl transferase (NMPRTase), a key enzyme involved in the biosynthesis of nicotinamide adenine dinucleotide (NAD) from the natural precursor nicotinamide. Intracellular NAD is essential for cell survival, and utilization of APO866 depletes NAD levels, thereby leading to cancer cell death. In pre-clinical studies, it was found that *in vivo* administration of APO866 as a single molecule inhibited tumor growth in animal models of human AML and lymphoblastic lymphoma without significant toxicity to the animals. These findings led to the initiation of a phase II clinical trial (NCT00431912). This study showed a moderately toxic effect of APO866 in cutaneous T-cell lymphomas [69]. Severe lymphocytopenia and thrombocytopenia were observed. Nevertheless, the drug did not show sufficient potency, ultimately leading to the study's discontinuation.

Methodical challenges in metabolomics

The advances in the study of metabolites over the past 20 years and the development of the metabolomics field have been driven by innovative improvements in scientific instrumentation and the increased availability of computational resources. It is widely recognized that a fundamental challenge in developing diagnostic methods lies in the requirement for standardization at every stage of the process. This includes defining selection criteria for the cohorts under study, specifying the metabolomics analysis methods employed, and culminating in the statistical analysis of the collected data. Implementing standardization across all these phases serves as a safeguard against the risks associated with inadequate control of metabolomics protocols, inaccurate quantification of metabolites, and potentially misleading data interpretation [70–72]. However, there are still pre-analytical, analytical, and post-analytical limitations that are hampering a wider clinical implementation of metabolomics.

The first area and core of any metabolomics experiment is data acquisition. Researchers unanimously emphasize the need to develop further research on standardizing procedures for preparing biological material for metabolomic studies [73, 74]. Vignoli et al. [75] showed a significant difference in metabolite concentrations between serum and plasma samples and between serum samples taken for different anticoagulants (EDTA and citrate). Several studies have confirmed that both exogenous factors such as smoking and ingested drugs, and endogenous factors such as ethnicity, gender, age, or even gut microbiota, have a profound impact on the results of metabolic assessments [76, 77]. Furthermore, any delays between collecting samples and their subsequent preparation can result in notable variations in the metabolomic profile. Improper storage and multiple freeze-thaw cycles can also compromise data quality [78]. Therefore, it is crucial to rigorously control and monitor the pre-analytical phase to prevent any adverse impacts on the profiles of the metabolome being studied. To achieve this, the entire workflow should be meticulously coordinated and guided by standardized operating procedures (SOPs) that are tailored for sample collection in omics methods.

Metabolites belong to different chemical groups i.e. lipids, amino acids, and sugars [3]. The simultaneous analysis of other classes of metabolites can be a technical challenge in performing a single test due to their chemical complexity and molecular heterogeneity.

Present-day metabolomics research primarily relies on two main analytical platforms for detecting, quantifying, and characterizing metabolites: 1) mass spectrometry (MS) — including liquid chromatography-mass spectrometry (LC–MS) or gas chromatography-mass spectrometry (GC–MS); and 2) nuclear magnetic resonance (NMR) spectroscopy [79]. NMR spectroscopy offers several advantages, including minimal sample preparation (it does not require extra steps such as separation or derivatization), high reproducibility, non-destructive analysis, and the detection of metabolites with varying physicochemical properties [80]. This allows for straightforward identification of discriminant

signals, resulting in less time required for sample analysis. However, NMR spectroscopy is limited by its relatively lower sensitivity compared to MS platforms [81, 82]. On the other hand, MS enables the study of various classes of metabolites at physiological levels and their accurate identification through fragmentation techniques. Moreover, compared to NMR spectroscopy, MS has the advantage of detecting smaller metabolites, with detection to the picomolar and nanomolar levels. Additionally, as various MS technologies have various operational principles (for example different ionization methods), the number of potentially measurable metabolites is expanding. However, due to the fact that MS is a destructive analysis, it is not possible to carry out several analyses on the same sample. Other drawbacks of NMR spectroscopy are its high cost in terms of acquisition and maintenance [83–85], lower level of reproducibility compared to NMR spectroscopy, the need for significant space for NMR instruments, and the requirement for highly skilled operators.

NMR and mass spectrometry are highly complementary, and combining these two techniques is likely to improve the overall quality of a study and enhance the coverage of the metabolome. While the majority of metabolomic studies use a single analytical source, there is a growing appreciation of the inherent value of combining NMR and MS for metabolomics. Metabolomic studies generate complex data with many parameters, and conducting a robust statistical analysis of the results is crucial to extracting meaningful insights. While web-based tools [86, 87] aid in processing and analyzing mass spectrometry-based metabolomics data for biomarker discovery and pathway enrichment, there is currently a gap in personalized metabolomics analysis tools. Most of the used statistical workflows are designed for case-control studies, and do not accommodate the inherent biological variabilities among individuals. Moreover, despite all these data-based advantages, the use and reuse of data from global metabolomics analyses in the frame of meta-analyses remain uncommon.

Metabolomics offers a comprehensive perspective on the current biological stage by examining changes in internal metabolites. As previously discussed, investigations into the impact of metabolic reprogramming in tumor biology have relied on collective metabolic analysis methods. Despite the advantage of having a larger quantity of material for analysis with bulk methods, single-cell metabolomics is essential for recognizing phenotypic variations within individual cells and uncovering subgroups of apparently similar cells to elucidate the origins of drug resistance, differentiation, or clonal evolution. One of the fastest developing branches of metabolomics in recent years has been single-cell metabolomics. Recent advances in such techniques promise to provide an unprecedented level of insight into hematological malignancies, shedding new light on their underlying causes. In the case of hematological cancers, researchers have mainly focused on distinguishing tumor cell heterogeneity using SCM. There have been studies assessing the differences between single AML cells [88], early-activated CD8+ T-cell [89], and plasma cells in MM [90].

Conclusions

The study of the metabolome of hematooncology patients can offer many potential diagnostic and therapeutic advantages at all stages of treatment. The main advantage of metabolic analysis over other systeomics branches is its ability to determine the current pathophysiological status of the disease [2]. Dynamically changing metabolite concentrations can indicate changes in the tumor clone at an earlier stage than current diagnostic methods. This enables the assessment of possible progression or resistance to treatment. Pathophysiological knowledge has expanded considerably recently and is a valuable theoretical prerequisite. Metabolomic profile evaluations predominantly rely on case-control study designs, typically featuring small patient groups. Furthermore, most studies have mainly focused on qualitative comparisons, whereas quantitative analyses have been lacking [21, 22, 25]. There is also a lack of results on the absolute concentrations of metabolic markers in tissues other than blood, particularly in bone marrow. Although many studies have described the diversity of metabolites associated with hematological diseases, the limited evidence for each metabolite suggests the need for further studies to confirm their potential as biomarkers.

This review article has discussed the most interesting reports on the use of metabolomics in hematooncology. However, there are a few limitations of our study. Firstly, given the extensive number of studies examining metabolomic profiles, we have chosen to discuss only a portion of the most significant research. Additionally, the paper only covered studies in the English language, but it is worth noting the substantial amount of research in other languages, particularly Chinese. We suggest that subsequent papers should focus on meta-analyses of research outcomes in specific diseases.

Metabolomics, being an interdisciplinary field, requires the integration of various disciplines, including analytical chemistry, molecular biology, biochemistry, and bioinformatics. The implementation of large-scale multicenter studies plays a crucial role in discovering the most promising pathophysiological pathways and further validating potential biomarkers, which may eventually lead to an increasing clinical application of metabolomics.

Article information and declarations

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Author contributions

JP — conceptualization. PH, KC — literature search and data analysis. First draft of manuscript written by PH and all authors commented on previous versions. All authors read and approved final manuscript.

Conflict of interests

The authors declare no conflict of interests.

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Supplementary material

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