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Altered Metabolism in Down Syndrome

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Abstract
Down syndrome (DS) is associated with aberrations in genetic, morphological, biochemical and physiological characteristics. A number of genes located on human chromosome 21 (HSA21) encode proteins which are thought to be involved in numerous metabolic pathways, e.g., phosphofructokinase, cystathionine β-synthase etc. Perturbations of the metabolic pathways may lead to altered drug metabolism in DS individuals. We present a review of metabolic aberrations linked to HSA21 genes in DS. We particularly focus on drug disposition, efficacy, sensitivity and toxicity of anti-leukaemic agents including methotrexate, glucocorticoids, anthracyclines and cytarabine in DS leukaemia. The different outcomes of therapy due to differential drug response, varied drug toxicity and treatment related mortality in DS leukaemia is a subject of much research and speculation. Altered drug response in DS individuals may stem from differences in pharmacokinetics, pharmacodynamics and pharmacogenetics. Further large-cohort studies in different age groups dissecting metabolic and molecular pathways involved in drug response may increase our understanding in this regard and stipulate pharmacotherapies in DS.

Keywords: Down Syndrome, Drug Metabolism, Pharmacokinetics, Pharmacodynamics, Methotrexate, Leukaemia, Glucocorticoids, Anthracyclines, Cytarabine

1. Introduction
Down syndrome (DS) is the most commonly reported genetic disorder characterized by intellectual disability which occurs in approximately 1/700 live births [1]. Despite advances in
antenatal screening and pregnancy termination, the prevalence of DS is increasing due to advanced maternal age and increased longevity of DS individuals [2-4]. Trisomy of all or part of human chromosome 21 (HSA21) is the underlying genetic abnormality that causes DS. Some 200-300 genes have been identified to be located on the long arm as well as on a portion of the short arm of chromosome 21 in DNA sequencing studies [5]. The overexpression of such genes likely results in cascading effects, eliciting interactions among gene products and between genes and environmental factors. These cause aberrations in the morphological, biochemical and physiological milieu of DS individuals resulting in the characteristic manifestations of DS.

Down syndrome Individuals exhibit altered metabolism which is attributed to the overexpression of some HSA21 localized genes or due to the presence of extra genetic information. A number of genes located on HSA21 encode enzymes those are thought to be involved in numerous metabolic pathways such as inositol, energy, cholesterol, choline, purine and reactive oxygen species pathways [6]. In this chapter, we review the literature for characteristic metabolic aberrations linked to HSA21 genes in DS. We particularly focus on the efficacy of chemotherapeutic agents such as methotrexate, glucocorticoids, anthracyclines and cytarabine in the context of drug disposition, sensitivity and toxicity in DS individuals since these agents form the backbone of current anti-leukaemic therapies.

2. Gene-dosage effects

Several genes involved in metabolism are located on chromosome 21 such as cystathionine \( \beta \)-synthase (CBS) gene encoding the CBS enzyme that catalyses homocysteine into cystathionine. Due to the presence of an extra copy of CBS gene in DS individual, lower levels of homocysteine and methionine are found in DS individuals compared to normal people which in turn leads to folate shortage and an altered metabolic state. Formimidoyltransferase cyclodeaminase (FTCD) is another gene located on the long arm of chromosome 21 which provides instruction to produce formimimotransferase cyclodeaminase enzyme involved in the metabolism of histidine and the production of folate required for synthesis of purine, pyrimidines and amino acids. Variations in the activities of various enzymes and plasma electrolyte concentrations to differ from normal parameters in DS have previously been reported especially in HSA 21 associated proteins such as S100B [7]. The levels of S100B protein were 4-8 times higher in DS individuals compared to the reference values. In addition, changes in metabolism of adenosine, homocysteine, purine and folate have also been reported [6].

Phosphofructokinase (PFK) is a key regulatory enzyme in glycolytic pathway as it catalyses the phosphorylation of fructose-6-phosphate to fructose-1, 6-bisphosphate [8]. Liver-type subunit of PFK (PFKL) is overexpressed in DS patients because its gene is located on chromosome 21 [9, 10]. Peled-Kamar et al. showed that transgenic mice overexpressing PFKL (Tg-PFKL) had aberrated glucose metabolism characterized by increases metabolic rate in brain and reduced clearance rate from the blood [11]. The enhanced glucose utilization observed in brain of Tg-PFKL mice is similar to faster cerebral glucose metabolism exhibited by young DS adults and may be linked to their cognitive disabilities. A previous study reported that PFK
specific activity is increased two-fold in the brains of embryonic Tg-PFKL mice [12] highlighting the fact that aberration in glucose metabolism are more pronounced in developmental period and may lead to DS associated learning disabilities. This observation of differential gene expression at different developmental stages further complicates the hypothesis of ‘gene-dosage effects’ in trisomy 21 consequently leading to varied metabolism aberration among different age groups. Further studies to determine metabolic variations in different age groups will not only increase our understanding in this regard but will also stipulate pharmacotherapies in DS.

3. Drug metabolism in Down syndrome

The effect of perturbations in metabolic pathways in DS is also reflected in the area of drug metabolism. Altered drug response has been reported in DS individuals compared to normal people, which may stem from differences in pharmacokinetics (PK) and pharmacodynamics (PD) in DS. Drug metabolizing enzymes, especially cytochrome P450, are a major source of variability in the PK of drugs. The CYP3A subfamily is believed to metabolize half of all prescribed drugs. Differences in the activity of cytochrome P450 may explain the altered drug response in DS individuals. We studied the CYP3A4/5 activity and found that children with DS had a 2.4 fold lower CYP3A4/5 activity compared to the children without DS (unpublished data). Alterations in PD have been reported for opioids, midazolam, acetylsalicylic acid and atropine [6, 13]. The metabolism of drugs is known to influence the active drug concentration of a drug, which either boosts or causes a reduced action of that drug. A drug which is subjected to increased metabolism will have a diminished intensity of drug action, as an increased metabolism will limit its duration of activity. On the contrary, a decline in the metabolism of the drug will intensify drug activity. Down syndrome individuals have a lower resting metabolic rate compared to normal people, which may contribute to altered drug metabolism and drug toxicity. Gut microbial chemical messengers regulate and influence host metabolism. Differences in gut microbiome may also be a relevant factor in altered drug metabolism in DS individuals and a review of literature is needed in this regard.

Down syndrome children are known to have an approximately 10-20 times higher risk of developing some blood cancers such as acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML) compared to non-DS children [14, 15]. In the last two decades, numerous studies have identified various drugs showing altered metabolism in DS patients in the context of childhood leukaemias. For instance, DS children suffering from AML have a better prognosis over non-DS children with AML. An in vitro study showed that DS-AML myeloblasts exhibited ten times higher sensitivity towards 1-beta-D-arabinofuranosylcytosine (ara-C), compared to myeloblasts from non-DS AML children [16]. On the other hand DS children with ALL show a pronounced intolerance for methotrexate which necessitates dose reductions and further adjustments in treatment protocols.

As described above, DS individuals have a high risk of developing haematological conditions, of which the most prominent is the development of acute leukaemias [17]. Acute lymphoid
leukaemia (ALL), acute myeloid leukaemia (AML) and a unique type of leukaemia exclusively associated with trisomy 21 called transient myeloproliferative disorder (TMD), commonly arise in DS individuals [18]. It has been recognized that while the presence of chromosome 21 trisomy on the one hand might be a deterrent against development of certain solid tumours in DS individuals, on the other hand it compounds the risk of development of haematological tumours [17]. Of note, is the fact that the acute leukaemias in DS shows marked differences from acute leukaemias in non-DS individuals. This varied picture reflects the underlying change, which ranges from molecular to systems level in DS individuals and confers unique characteristics to host and disease biology including marked differences in disease outcomes as compared to non-DS leukaemia patients. The different outcomes of therapy due to differential drug response, varied drug toxicity and treatment related mortality (TRM) remains in DS leukaemia a subject of much research and speculation. In this connection, various studies in the last two decades have highlighted the direct or indirect involvement of the extra copy of chromosome 21 with its gene dosage imbalances as a basic factor [19]. Additionally, acute leukaemia is known to be a highly heterogeneous disease [20, 21]. In DS patients with their characteristic biochemical and pathophysiological state it most likely contributes to the distinctive malignancy pattern observed in them.

Recent studies have identified new subtypes of DS leukaemia patients [22, 23]. The subtypes show distinctive prognostic features which also have a bearing on event free survival (EFS) rates. This brings into perspective the issue of present drugs commonly used for the treatment of leukaemia in DS, their metabolism and treatment outcomes. In this respect, studies linking the genes or possible candidate genes involved, the molecular pathways influenced by these genes, the gene products, the pathophysiologic milieu of DS leukaemia and the pharmacological differences emerging thereof have begun to clarify the drug sensitivity, drug responsiveness and drug toxicity profiles in the setting of DS leukaemia. This has resulted in protocol modifications in certain cases of DS leukaemia and improved treatment outcomes [24, 25]. Patients’ response to a particular therapeutic regimen and the drugs concerned happens to be the most crucial prognostic predictor of treatment outcomes. In this context both malignant cell genetics and patients’ pharmacodynamics and pharmacogenetics are intimately involved [26].

Studies in drug pharmacodynamics and pharmacokinetics have been helpful in informing and formulating treatment regimens in DS and NDS leukaemia patients. Pharmacogenetics and pharmacogenomics deals with the effect of a particular gene or genome wide associations respectively on drug response of an individual [27]. Pharmacogenomics is a relatively new and promising area which has the potential to unravel the enigmatic issues associated with DS leukaemia. Altered metabolism of drugs is well recognized in cancers including leukaemia and DS leukaemia [28-30]. High to severe drug related toxicity in ALLDS patients is a known phenomenon. The toxicities may be related to the gastrointestinal mucosa, infectious toxicity secondary to MTX use, haematological toxicity and hepatic toxicity, which under severe circumstances may lead to liver fibrosis and neurological toxicity [31]. Besides, ALLDS patients exhibit a higher level of resistance to certain drugs used in various chemotherapeutic regimens than ALLNDS individuals.
In DS individuals, more data are present for anti-leukaemic agents than any other drug. Therefore, we present a detailed review of methotrexate, glucocorticoids, anthracyclines and cytarabine in the next section.

4. Methotrexate

Methotrexate (MTX), an anti-folate agent [19] is one of the most widely used and frequently studied drugs in various malignancies including leukaemia [22]. In ALL, the drug is usually administered as high dose MTX (HDMTX) which constitutes 500mg/m² and above. It is an anti-metabolite that inhibits cellular growth by obstructing the formation of purines and thymidine phosphate. MTX is known as a competitive inhibitor of dihydrofolate reductase (DHFR), an enzyme required for the conversion of tetrahydrofolate from dihydrofolate in dividing cells. MTX competitively binds to DHFR, replacing dihydrofolate. This results in the lack of regeneration of tetrahydrofolate, a reduced folate that is essential for de novo purine and thymidine phosphate synthesis. This eventually blocks DNA synthesis [32-34].

MTX entry into the cell is facilitated by the reduced folate carrier (RFC) protein, which also transports dihydrofolate, 5-methyl tetrahydrofolate and folinic acid [35]. The gene for RFC protein is localized on chromosome 21q22, so an increased gene dosage effect as a result of chromosome 21 in DS, likely leads to entry and accumulation of higher amounts of MTX with subsequent formation of MTX polyglutamates. The increased accumulation of MTX polyglutamates in cells can be a marker of MTX toxicity [36]. Since every somatic cell in DS patients has an extra copy of chromosome 21 (constitutional trisomy), increased MTX absorption in ALLDS, especially in the GI tract, may be responsible for the severe toxicity to methotrexate observed in such patients [28, 30]. Polymorphisms in several genes concerned with folate metabolism and their association with MTX-generated toxicity have been identified. A retrospective study of 81 ALL children who had received treatment in keeping with the Dutch Childhood Oncology Group (DCOG) ALL-9 protocol, was conducted by Huang et al. The therapeutic regimen included high dose methotrexate (HDMTX) administration continuously as an IV infusion for a 24-hour period followed by leucovorin rescue in three doses. The results indicated that patients with methylenetetrahydrofolate reductase (MTHFR) 1298 AC and CC and serine hydroxymethyl transferase (SHMT) 1420 CT genotypes showed less toxicity to MTX, whereas methionine synthase reductase MTRR 66 AG and GG genotypes exhibited higher toxicity [37].

A spectrum of side effects may arise from methotrexate use. Patient-to-patient variations have also been recognized. The common side effects with increased frequency and severity in DS individuals are mucositis, nausea and vomiting, diarrhoea, myelosuppression and hepatic toxicity marked by perturbations in liver enzymes (transaminases). Coagulation and pancreatic toxicities have also been reported after augmentation of methotrexate dose in post-induction phases [38]. Additionally, central nervous system (CNS) toxicity, which may precipitate in learning disabilities of a more complex and intractable nature in ALLDS than in AMLDS, has been identified [39].
An important strategy to reduce MTX toxicity is the administration of folic acid (Leucovorin) usually between 24 and 36 hours after administering HDMTX. Leucovorin selectively rescues the normal cell from the adverse effects of methotrexate by restoring reduced folates in the normal cells so that they use it in the formation of purines and thymidine phosphate [40]. In an earlier study, Peters and Poon described methotrexate sensitivity in four patients with DS leukaemia who had received MTX in the course of treatment [41]. Severe and immediate toxic effects such as rash, diarrhoea and mucositis were detected in them irrespective mode of drug administration i.e., intravenously, intrathecally or orally. The drug became tolerable after dosing was considerably reduced (30%-50%). However, methotrexate absorption and clearance was within normal parameters in the two patients who were evaluated for it. The authors suggested the possible involvement of enzymes synthesized by genes on chromosome 21 related to purine metabolism such as tetrahydrofolate [41]. In the Medical Research Council (MRC) UKALL XI study most of the children with ALLDS did not display an unusual toxicity during the course of HDMTX therapy [42]. The authors attributed this to stringent adherence to protocols of leucovorin rescue. However, no comparison with ALLNDS children was made.

The pharmacokinetics of methotrexate in ALLDS was described by Garre et al. in a study designed to determine the frequency and severity of MTX toxicity in five DS ALL children, and the results were compared with NALLDS children [43]. ALLDS patients showed significantly higher toxicity in spite of having received relatively larger doses of leucovorin in anticipation of higher toxicity. Gastrointestinal toxicity was the most prominent and at high degrees (grade 2-4). Other MTX-related toxicities noted were myelosuppression up to grade 4, CNS symptoms, hepatotoxicity and maculopapular rash on skin. Repeated leucovorin in high doses may have lessened certain incidences of high toxicity to some extent but did not prevent toxic manifestations altogether. Furthermore, leucovorin reduced the occurrence and severity of mucositis, the most common feature associated with MTX toxicity, in all but one patient. In this patient, MTX doses were subsequently reduced which brought down gastrointestinal and haematological toxicities to lower grade. Drug pharmacokinetics study showed that DS patients had a greater plasma MTX concentration at 42 hours after initiating MTX therapy in comparison to ALLNDS patients which signifies altered metabolism of the drug. However, plasma MTX clearance did not materially vary between the two groups. A sevenfold risk of MTX toxicity was found to be associated with ALLDS patients. This study points to the possibility of enhanced tissue sensitivity to MTX as well as variation in drug pharmacokinetics between ALLDS and ALLNDS patients. However, a recent retrospective case controlled study of 44 ALLDS patients did not detect any clinically significant variation in the pharmacokinetics of MTX between ALLDS and ALLNDS patients. [32]. The MTX clearance rate was slower by 5% in ALLDS than in ALLNDS patients but the investigators did not deem it to be of clinical importance as, at both 24 and 48 hours, the MTX plasma concentrations between the two groups did not differ widely. As in other previous studies, this investigation also recorded a very high number of ALLDS patients displaying grade 3-4 gastrointestinal toxicity. The incidence of toxicity remained higher than that of ALLNDS patients even after dose-lowering regimens were put into effect. Patients who had received doses of mercaptopurine during MTX treatment did not present with any blood related toxicity. This study [32]
strongly suggests the role of differential MTX pharmacodynamics, especially in relation to the gastrointestinal mucous epithelium of the two patients groups, and highlights the possible differences in the uptake and subsequent accumulation of MTX and MTX polyglutamates (MTXPG). It seems pertinent to mention here that MTXPG remains in cells for a longer period, and increased polyglutamation could result in cellular injury and destruction of the cells of the intestinal mucosa. This may likely explain the exacerbated MTX toxic manifestations in ALLDS patients [32]. Additionally, germline polymorphisms in candidate genes may play a role in influencing the action of drug-related enzymes, proteins and drug targets, which may subsequently contribute to enhanced MTX toxicity [44]. Children with more polymorphisms have more gastrointestinal mucosa-related toxicity and hence may show altered pharmacodynamics [31].

The Ponte di Legno (PDL) study [22], the largest retrospective study to date, was conducted to explore the features of ALLDS; the data of 653 ALLDS patients who were enrolled in various international studies were analysed. It emerged that the cumulative incidence of relapse (CIR) was higher compared to that of ALLNDS patients. Moreover, the two-year period of treatment-related mortality (TRM) was higher in ALLNDS patients. These characteristics were seen to have a negative impact on the eight-year event-free survival (EFS) and overall survival (OS) of DS patients. This and several other studies compel us to further investigate the possible role of heightened MTX toxicity and its ramifications in ALLNDS individuals with regard to treatment outcomes, including TRM, EFS and OS. This remains a complex issue at best, as not only the unique constitutional patient characteristics of DS but the characteristics of leukaemic cells in the setting of DS must be considered. Additionally, interactions between MTX and other drugs that are simultaneously given to patients may complicate the issue further. Therefore, there appear to be multiple mechanisms and processes that regulate the response to this drug [19]. The literature is replete with instances where, due to toxicity-related issues, treatment protocols have been modified in which, more often than not, a reduction in MTX dose has been made. Does it affect the natural history of disease in ALLDS patients and result in poorer outcomes? The PDL study concluded that the largely dismal prognosis of ALLDS is chiefly the result of a higher rate of relapses, and TRM is a less significant factor. In light of these findings, the study does not recommend treatment reduction in general. However, for a sub-population of patients with high hyperdiploidy or ETV6- RUNX1 mutations in which toxicity is a leading cause of death, treatment modifications including drug reduction may be opted [22].

The issue of dose reduction in ALLDS in the face of potential MTX toxicity has another aspect, i.e., an overcautious approach on the part of the treating physician leads to a reluctance to use appropriate doses of this drug. A study in DS children found that physicians used MTX and 6-mercaptopurine doses at a lesser concentration than what is prescribed in both standard protocol treatments and is also given to ALLNDS patients [45].

Significant neurological toxicity is associated with MTX use [32]. Fortunately, most of the symptoms are transient and resolve quickly, or resolve at least at the end of therapy [46]. However, there are several other studies that have investigated the long-term effects of MTX.
on CNS [39, 47, 48]. For ALLDS children cranial irradiation is not prescribed by any of the cancer study groups. Therefore, in such patients the effect of chemotherapy, including methotrexate therapy, on the CNS and its long-term sequelae remains a vital area for the conduction of large-scale studies. As overall survival of ALLDS patients has improved, the issue of quality of life assumes much importance. Inherently compromised brain functions unrelated to leukaemia are a feature of DS. In this setting, coupled with a higher overall toxicity to MTX, ALLDS children have a greater risk of chemotherapeutic insults to the brain with the possibility of worse long-term neuropsychological sequelae including learning disabilities and emotional problems. Intrathecal as well as intravenous MTX administration may be associated with leukoencephalopathy, cerebral cortex atrophy and seizures [48, 49]. Furthermore, an earlier study has reported that intrathecal cytosine arabinoside can augment the CNS toxicity of MTX [50]. A recent study has reported the detection of extensive vascular myelopathy of the spinal cord on an autopsy of an ALLDS patient who was given MTX therapy. The authors suggested a possible role of MTX in the processes of white matter degeneration [51]. Krull et al. found evidence of a direct effect of MTX on neurocognitive functions in ALL patients who were alive ten years or more after diagnosis [47]. After controlling for cranial irradiation it was determined that each 1gm/m² of MTX aggravated the possibility of slowed mental processing speed by 3% [47].

MTX administered in escalating doses with the initial dose of 100mg/m² and gradually raising it by 50mg/m² without following it with folinic acid rescue, until the moment toxicity is detected is also known as Capizzi MTX. This method is known to be associated with superior outcomes in standard risk ALL [38]. Larsen et al. in a study compared HDMTX with leucovorin rescue and Capizzi MTX in children, adolescents and young adults treated in accordance with the high-risk COG ALL protocol [52]. Better EFS was reported in the HDMTX arm than in the Capizzi MTX arm. Furthermore, fewer incidences of treatment failures including marrow and CNS failures were observed in the HDMTX arm. In the context of ALLDS patients, the mere extrapolation of these results may not be of much help to draw any clinically definitive conclusion. Clearly, there is a need to conduct clinical trials involving DS patients which could help in the way of building up a more personalized approach to treatment of DS patients. Recently, an uncommon case has been reported of a child with ALLDS who was earlier a patient of AML, who was treated successfully and remained in remission from AML when ALLDS was diagnosed at four years of age. In an intensified consolidation regimen, he was treated with an escalating dose of MTX (Capizzi MTX) given intravenously, which was not followed by leucovorin rescue. Following the second Capizzi MTX (100mg/m²), the child developed mucositis of moderate severity and was subsequently put on a low risk maintenance regimen which had to be curtailed from three to two years. This modification fortunately had no unwanted clinical effect as the child remained disease-free until the time last reported, i.e., at seven years post-diagnosis [30].

As in the case discussed above, and in the majority of DS patients treated with MTX, mucositis emerged as the leading toxic manifestation. The intrinsically unique immunometabolism in DS coupled with the rather compromised aspect of certain components of the immune system
could also be a contributory factor in mucositis. Combined with this, the effect of MTX metabolism in the setting of DS and enhancement in apoptosis of the mucosal cells breaches the cellular barriers resulting in higher grades of mucositis [30]. In a study performed to explore the metabolic and genetic components responsible for MTX toxicity, 134 childhood ALL patients were treated in line with the DCOG-ALL-10 protocol [53], and the results showed that MTX-associated mucositis was more common and frequent mainly after the first course of the drug. Besides, mucositis also accounted for the major share of overall drug related toxicity (> 3). However, since the patients had neutropenia, and, prior to starting the MTX therapy, had received drugs such as mercaptopurine, cyclophosphamide and cytarabine, the concomitant role of these drugs in aggravating the moderately severe mucositis cannot be completely ruled out [54, 55]. This might explain the higher toxicity at the end of first MTX course, since the second MTX course was not preceded by these drugs. This observation raises the possibility of interaction between drugs and their cumulative side effects, which should be further explored in every subtype of ALL including ALLDS. Since leucovorin rescue was initiated 42 hours after the commencement of the first HDMTX dose, its detoxifying effect is most likely to build up in the later phases of treatment and not in the starting phase, which also could have contributed to a higher toxicity encountered during the first course. The PDL study found that apart from reducing doses of MTX, leucovorin at a high dose was given to patients with ALLDS by the various study groups treating ALLDS patients [22]. It is plausible that a higher leucovorin dose might interfere with the efficacy of MTX by rescuing the cancer cells in addition to normal cells in ALLDS. This attenuation in the effect of MTX may have important implications for therapy and therapeutic outcomes. Several studies have shown that leucovorin in higher concentrations or its fairly early dosing after MTX administration may increase the risk of disease relapse [56, 57]. Skarby et al. studied the associations between disease relapse, serum methotrexate concentrations and leucovorin rescue doses in 445 children with ALL who were treated according to the Nordic Society of Pediatric Hematology and Oncology (NOPHO) ALL92 protocol [58]. Higher leucovorin doses matched with a corresponding higher MTX (which was determined by high serum MTX); the results indicated that the attempt to put into effect a heightened leucovorin rescue compromised methotrexate efficacy. Disease relapse risk was aggravated, registering a 22% increase when the leucovorin dose was doubled. This study led to the modification in the NOPHO protocol, which then prescribed a reduction in leucovorin dose. To our knowledge, no study similar to this has been conducted in ALLDS patients; however, since the ALLDS patients continue to be treated with minor modifications of the existing protocols for ALLNDS this study also holds relevance for these patients. Leucovorin doses in many instances have been intensified for ALLDS patients. Therefore, separate studies in ALLDS patients are needed to determine the impact of higher doses of leucovorin on the efficacy of HDMTX. Question remains about the optimum leucovorin dose that on the one hand would keep toxicity within tolerable limits and on the other hand would not dilute the effect of HDMTX. The enzyme dihydrofolate reductase is believed to mediate in the competitive activities of these two antagonists, i.e., MTX and leucovorin [59]. Complicating the matter is the fact that cancer patients are prescribed folate as part of a nutrient supplementation in order to increase appetite and reduce anorexia. This and the dietary folate may also influence
the therapeutic effectiveness of HDMTX along with leucovorin. Studies using modern sequencing platforms may yield further information about genetic variations in enzymatic interactions and drugs that will aid in reaching a meticulous balance between MTX dose and leucovorin strength. It will also lead to a more personalized approach, the significance of which cannot be overstated in ALLDS given the heterogeneous nature of the disease [60].

The Ponte di Legno study concluded that disease relapse in ALLDS patients was the foremost cause of inferior survival. In the light of this finding it does not recommend any reduction in treatment except for a small minority genetic sub-group with ETV6-RUNX1 or high hyperdiploidy, where toxicity-related mortality was identified to be the highest. Interestingly, this sub-group is also associated with the most favourable prognosis and a very low cumulative incidence of relapse [22]. In recent years there has been a marked improvement in EFS and OS of ALLDS children and also less disease relapse due to relatively fewer induction deaths and treatment-related mortality as compared to the pre-2000 era [61]. This is likely in part due to the emphasis laid upon not decreasing treatment intensity. Buitenkamp et al., for instance have shown that usage of MTX in intermediate doses does not lead to any unexpected major toxicity issue in ALLDS children. The authors advised that 1-3g/m² of MTX administration coupled with meticulous observation for any unwanted toxicity should not be unsafe as a starting regimen [32]. Advances in supportive care for these patients might further improve the end results of the use of chemotherapeutic drugs including MTX.

The current treatment strategies for ALLDS patients entail a modification of treatment keeping in view the objective to limiting toxicity and minimizing treatment-related mortality. Needless to say, this is expected to have a positive impact on disease relapse as well as on the overall quality of life. In ALLDS patients the best practice is that MTX is intravenously administered in the range of 500-1000mg/m² with leucovorin rescue and no further augmentation of dose. The other strategy is to adopt a dose escalation regimen, starting with 500mg/m² and gradually escalating to 2000mg/m² (Capizzi MTX). In high-risk ALLDS patients, dose reductions of HDMTX should not be resorted to, and Capizzi MTX administration, where protocol dictates, should not be halted or modified in the face of apprehensions of infection. As discussed earlier, dose reductions can be made in the favourable prognosis sub-group as well as those in which minimal residual disease shows negative results [30].

5. Glucocorticoids

Glucocorticoids (GC) such as prednisolone and dexamethasone along with L-asparaginase constitute an important component of therapy for all types of ALL patients, including ALLDS patients [62]. However, usually transient hyperglycaemia has been associated with the use of glucocorticoids and L-asparaginase and studies have indicated a synergistic role of glucocorticoids and asparaginase in the development of this condition in leukaemia patients [63]. In ALLDS patients the risk of hyperglycaemia is compounded, which necessitates a more thorough observation and prompt remedial measures. In the St. Jude hospital study, Pui et
al. showed that age, obesity and DS were each linked with a higher risk of hyperglycaemia in patients who received treatment with L-asparaginase and prednisone [64]. Additionally, in circumstances where all these traits appear together, a combinatorial effect on glucose intolerance could be witnessed. The authors also highlighted the relatively-increased blood glucose levels in hyperglycaemic DS patients as compared to non-DS patients exhibiting hyperglycaemia due to treatment [64]. Similarly, earlier studies had reported a high incidence of non-ketotic hyperosmolar diabetic coma leading to high mortality in DS children [65, 66].

It is well established that DS patients have a greater propensity to develop diabetes mellitus, and that too relatively earlier in life [67-69]. This has made DS an independent risk factor for a hyperglycaemic dysmetabolic state during the course of glucocorticoid therapy in ALLDS individuals [22]. A glucocorticoid-mediated hyperglycaemic dysmetabolic condition could be a contributory factor in the poor prognosis associated with ALLDS patients. Altered metabolism of glucose in ALLDS leukaemia is most likely a contributory factor that influences disease prognosis. No study in ALLDS has provided direct evidence in this regard. However, Boag et al. showed that non-solid tumours (pre-B ALL cells) exhibit alterations in their metabolism such as switching to exacerbated aerobic glycolysis and an increase in the number of glucose transporter GLUT 1 [70]. The authors stated that apart from tumourigenesis, the course of disease and its prognosis may also be associated with metabolic changes. A recent study has shown that dexamethasone inhibited the entry and utilization of glucose and caused disruption of glycolysis, resulting in cellular death in primary ALL blasts and ALL cell lines [71]. Furthermore, higher apoptosis was identified in those cells in which glucose concentration was relatively low, which reveals that an efficient and higher apoptotic rate could be reached under lower glycaemic conditions. These studies should be reproduced in ALLDS individuals and results thus obtained would help better address the issue of hyperglycaemia in ALLDS as altered metabolism is generally more common in DS [17, 28, 30], also, in the light of the findings that glucocorticoid sensitivity could play a critical role in influencing treatment outcomes and the increased resistance to these hormones could lead to a worsening prognosis. Holleman and colleagues have described that genes related to glucose metabolism are highly expressed in the cells resistant to glucocorticoids [72]. Notably this was detected in patients with pre-B ALL cells, and the overwhelming majority of ALLDS belongs to the pre-B cell phenotype [18]. Moreover, enhanced glycolysis in cells exacerbates the risk for glucocorticoid resistance in leukaemic lymphoblasts [73]. Identification and targeting of the upregulated genes and the genetic pathways involved in the generation of higher glycolysis in DS patients in future may prove to be advantageous.

Prednisolone and dexamethasone have both been in use in ALL for decades, especially in the remission induction phase of therapy. However, studies have indicated better treatment results with the use of dexamethasone, especially in the context of the higher efficacy of dexamethasone in penetrating the blood brain barrier, leading to a lower rate of CNS relapse [74-77] and meningeal leukaemia [78]. However, dexamethasone use is associated with a higher drug toxicity and a higher rate of infections. Higher infections, partly as a result of therapy causing myelosuppression, is well known in DS patients. Recently, Domenech et al.
have shown that dexamethasone at 6mg/m²/day and prednisolone at 60mg/m²/day exert equal benefits and that no significant variation in toxicities have been detected in the use of these two drugs at the tested dosing [79]. However, the majority opinion seems to be that dexamethasone is a better choice as far as improved CNS control is concerned. Enhanced steroid toxicity could also manifest as myopathy and a marked increase in weight. DS individuals independently show a predilection towards more weight gain [17]. Which of the two drugs, dexamethasone or prednisolone, can lead to greater weight gain or exert an equal effect is a moot question. Genetic profiling of patients in the context of glucocorticoid activities combined with metabolomic studies may give some direction in the future. Very recently, Bindreither et al. studied the transcriptional profile of T-ALL cells after treating them with prednisolone and dexamethasone, which showed no remarkable variations in the transcriptional responses detected [80]. These results also highlight that both of these glucocorticoids regulated identical genes. Furthermore, the authors conclude that the differential treatment outcomes of dexamethasone and prednisolone, as reported in several studies, are perhaps due to differences in the pharmacokinetics and pharmacodynamics of these drugs. Studies dissecting the metabolic and molecular pathways involved in the glucocorticoid response in DS patients will help to inform practitioners to adjust and improve therapy. As of now, the point made by Inaba and Pui holds relevance that, in view of the fact that considerable variations are encountered among patients with regard to the sensitivity of ALL cells, and the same for glucocorticoid toxicity, best practice would be to consider the risk of relapse, phase of therapy and the drugs that are administered concomitantly with the glucocorticoids, before opting for a particular glucocorticoid [62]. Currently, in delayed intensification therapy for ALLDS patients, MRC UK and COG cancer groups recommend discontinuous dexamethasone dosing [18, 30].

6. Anthracyclines and Cytarabine (Ara-C)

Anthracyclines, mainly daunorubicin, doxorubicin and idarubicin, are used in the therapeutic regimens for treating ALL as well as AML [15, 81]. These anti-tumour antibiotics bind to DNA and prevent the unwinding activity of topoisomerase, which leads to abrogation of the process of DNA replication. Cytarabine (ara-C) incorporates into DNA through its active metabolite and impedes the binding of d-CTP to DNA. It abrogates the activity of the DNA polymerase enzyme [28]. In current treatment strategies, ara-C has taken the central stage for AML treatment, including AMLDS.

In ALLDS patients, anthracycline use is advocated differently by the groups. The Dutch Children Oncology Group (DCOG) and France Acute Lymphoblastic Leukaemia (FRALLE) do not use anthracyclines for induction therapy at all, whereas, daunorubicin induction is given by some groups in the case of patients with inadequate response. Anthracycline, along with glucocorticoids were given in the induction phase by physicians at St. Jude Research Hospital, fortunately with no reported unexpected toxicities. For further improvement in survival and lowering toxicities especially cardiac toxicity, further trials and research studies
are greatly needed so that the optimum balance of modern therapy can be reached in the different risk groups of patients with ALLDS [18].

Several studies have been conducted in AMLDS patients to determine the effects, side effects and efficacy of anthracycline, which also highlights the altered drug response and unique genetic and metabolic make up of AMLDS individuals. Unlike ALLDS, AMLDS children have a better outcome with the highest curative rate of any other group of myeloid leukemia patients [16, 82, 83]. The French American British Classification (FAB) classification AML M7 is characterized by one of the worst prognoses in children without DS. However, the situation is different in the context of acute megakaryoblastic leukemia (AMkL) DS, especially in children younger than three years of age, where excellent prognosis in recent years has generally been acknowledged [18, 84]. AMkL happens to be the most common phenotype in AMLDS [19].

Increased sensitivity to anthracycline and other drugs can be attributed to the presence of a high level of oxygen free radicals which are inherent in the cellular constituents of DS individuals [28, 85]. The enhanced ROS production coupled with perturbation in superoxide dismutase observed in DS, in the absence of concomitant increase in other antioxidant enzymes such as catalase and glutathione peroxidase, predisposes the cells to undergo apoptosis. In this altered metabolic state, drug-induced apoptosis is further exaggerated. It has been proposed that the HAS 21-linked gene dosage effect of NADH dehydrogenase ubiquinone flavoprotein 3 may be responsible for increased ROS production through enhanced mitochondrial respiration [84]. Reduced anthracycline dosage is now universally prescribed for AMLDS patients, especially to obviate the well-known cardiotoxicity associated with anthracycline therapy. This is particularly beneficial to DS individuals as they are prone to mitochondrial dysfunction and thereby increased cardiac oxidative stress [86].

The well-known favourable prognosis in AMLDS children reflects the enhanced sensitivity of leukaemic blasts to cytarabine (ara-C) and anthracyclines [87-89]. The modification of cancer drug metabolism including ara-C in AMLDS individuals has been the subject of extensive research. A landmark study by Taub et al. found that DS myeloblasts showed an enhanced sensitivity to ara-C, which was ascribed as being a contributory factor to better prognosis. This is further supported by the observations that both DS myeloblasts as well as trisomy 21 lymphoblastoid cell lines accumulate higher ara-CTP (a metabolite of ara-C synthesis) levels than non-DS cells. Taken together, these observations imply an altered metabolism of ara-C in DS children. However, other factors like the concomitant use of daunorubicin or the gene dosage effect of enzymes related to chromosome 21 such as carbonyl reductase and superoxide dismutase (SOD) may also influence outcomes in AMLDS patients [16]. Zwaan et al. showed that AMLDS cells were 12 times more sensitive to ara-C than AMLNDS cells [89]. The authors also determined a two- to seven-fold heightened anthracycline sensitivity in AMLDS cells. In another study, AMLDS cells showed a several-fold increase in sensitivity to both ara-C and daunorubicin in MTT drug assays. Besides, a remarkably higher concentration of ara-CTP was also detected, which strongly pointed to a link between trisomy 21 and high drug sensitivity [90]. Other studies have strengthened this view and implicate an altered metabolism of both ara–C and daunorubicin in AMLDS children [88, 89].
Mutations in the GATA1 transcription factor gene present on chromosome X is an exclusive feature of AMkLDS [29]. These mutations likely contribute to augmented ara-C sensitivity. GATA1 mutants were found to have a truncated 40 KDa protein in AMLDS blasts instead of the wild-type 50 KDa protein [29]. This affects cytidine deaminase (CDA) gene expression, which is believed to result in less CDA expression in DS myeloid blasts, accounting for the increased ara-C sensitivity. Another factor that might play a role in increased ara-C sensitivity is the alteration in folate metabolism, which is traced to the heightened activity of the CBS enzyme linked to HSA21.

7. Future directions in pharmacotherapy

With an increased longevity of DS individuals, new challenges have arisen in the area of the management and treatment of associated morbidities. An increasing age carries the potential to modify the natural history of the disease. Additionally, in DS the phenomenon of accelerated ageing, and thereby accompanied changes in metabolism, is a factor that can hardly be ignored. A better understanding of drug metabolism in DS is extremely useful because DS individuals receive several medicines as part of their palliative care and to combat concomitant illnesses. Further collaborative studies in a larger DS cohort are warranted to better understand the phenomenon of altered drug metabolism and concomitant drug interactions. DS is characterized by phenotypic heterogeneity, and the varying severity and complexity of the disease involves multi-organ systems. Given the wide spectrum of clinical anomalies in DS, future pharmacotherapy approaches need a more tailored approach for personalized medicine based on advanced knowledge and input from drug metabolism studies in DS individuals. Thus, further research and information from case studies and drug trials are warranted to adopt appropriate treatment regimes. It may also be relevant to neurodegenerative diseases such as dementia and Alzheimer’s, which have an earlier onset in DS and are also prevalent in a large proportion of elderly people in the general population.

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