The Effect of Methotrexate Treatment on Neuroinflammation Gene Expression in Pediatric Cancer Patients

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Background

Pediatric cancer survivors often experience long-lasting health consequences from their chemotherapy treatment. These deficits, termed late effects, are sequela that arise months or years after a disease is identified or treatment has been completed.

Various types of therapy include:

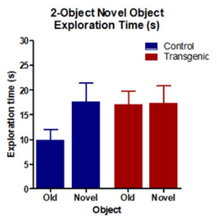
- Surgery
- Bone marrow transplantation
- Radiation therapy
- Chemotherapy

Figure 1: White allows different parts of the brain to communicate across long distances

Results found in literature:

- Mice in chemotherapy group showed impaired learning versus controls
- Glial cell histology indicated differences in white but not grey matter in mouse and human tissue.
- White matter contains few cell bodies and primarily long-range myelinated axons.
- Decreased myelinization may underpin learning impairment via interruption of normal oligodendrocyte and other glia development.

Figure 2



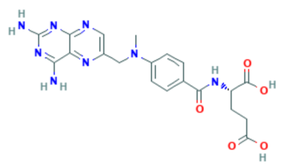
Methotrexate

The chemotherapy agent most used for the treatment of pediatric leukemias, and lymphomas is methotrexate, a synthetic folic acid antagonist.

MTX indirectly inhibits cell division through the blockage of folate-related enzymes.

Methotrexate treatment in pediatric patients has been associated with the long-lasting development of detrimental neurological and psychosocial sequela following cancer survival.

Figure 3: Chemical structure of methotrexate



Neurocognitive and psychosocial late effects

Neurocognitive and psychosocial late effects of chemotherapy: occur in 40-60% of acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML) survivors.

The Late Effects Clinic at Children's Hospital in New Orleans: monitors cancer patients two or more years after completing treatment or five years after diagnosis.

Neurocognitive deficits associated with cranial irradiation and methotrexate treatments may cause patients to struggle with the expectations of the classroom/school and in keeping up with peers academically.

Specific neurological issues found in literature include problems with:

- Communication
- Slow processing speed
- Trouble with fine-motor skills
- Multitasking
- Executive functioning– skills such as managing time, paying attention, shifting focus, organizing, and memorizing details

Psychosocial problems reported in the literature were abnormal behavior, emotional problems such as unusual aggression, lack of self-confidence, poor social skills, and trouble relating to peers.

Project goals

Hypothesis: methotrexate treatment causes abnormal gene expression in neuroinflammation genes in the white matter and changes in neurological and psychosocial development in children.

Goals: Provide information regarding gene-environment interactions and thus reveal candidate risk genes and pathways contributing to neurocognitive and psychiatric late effects. Our research is incredibly important because the evaluation of genetic differences in cancer patients has not previously been evaluated. The long-term goal of this project is to improve the health outcomes and quality of life of cancer survivors.

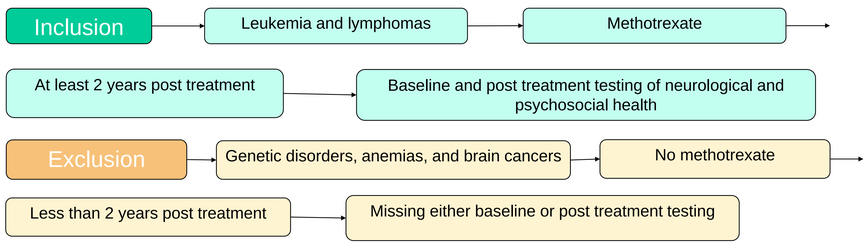
Specific aim 1: Retrospective study of cancer survivors

- The patient charts will be reviewed retrospectively from the Late Effects Center at Children's Hospital of New Orleans, LA (CHNOLA).
- The clinic follows patients two years post-chemotherapy to evaluate any changes in neurological, visual, hearing, learning, motor skills, and other developmental outcomes up to the age of 22 years.

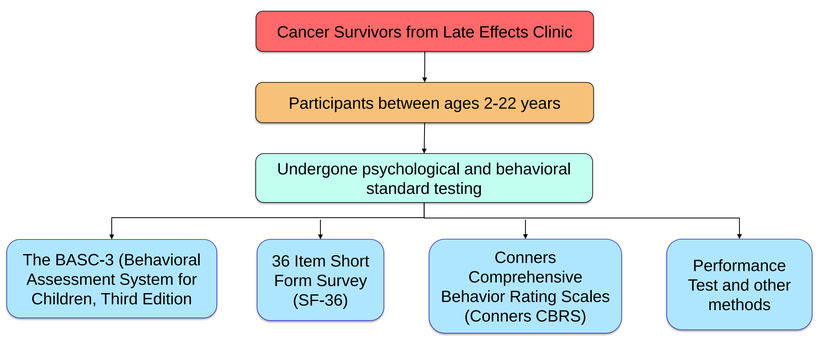
Specific aim 2: Genetic analysis of deceased cancer patients

- Evaluation of gene expression of known neuroinflammation genes on white matter samples from deceased patients who received methotrexate.
- Epidemiological data will be obtained from the patient charts to determine information regarding age of treatment, type and severity of cancer, length of treatment, and neurological and psychosocial effects.

Specific aim 1: Retrospective study of cancer survivors



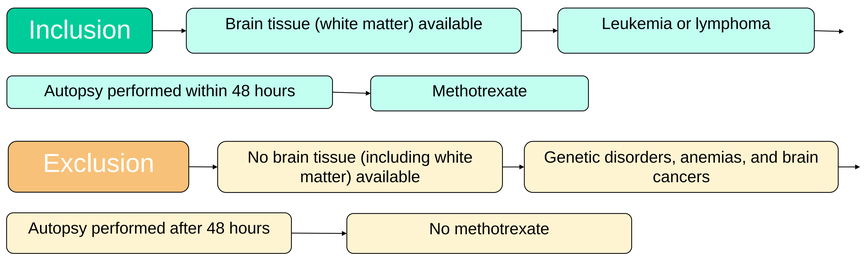
Methods



Preliminary Data from the Late Effects Clinic (n ≥ 100)

Adverse Outcome	Therapeutic exposures associated with increased risk	Factors associated with highest risk
Psychosocial effects	Any cancer experience	CNS tumors Cranial irradiation Hearing loss Older age at diagnosis
Neurocognitive deficits	Methotrexate (intrathecal, high-dose IV) Cranial irradiation	Intrathecal methotrexate Female sex Younger age at treatment Cranial irradiation

Specific aim 2: Genetic analysis of deceased patients



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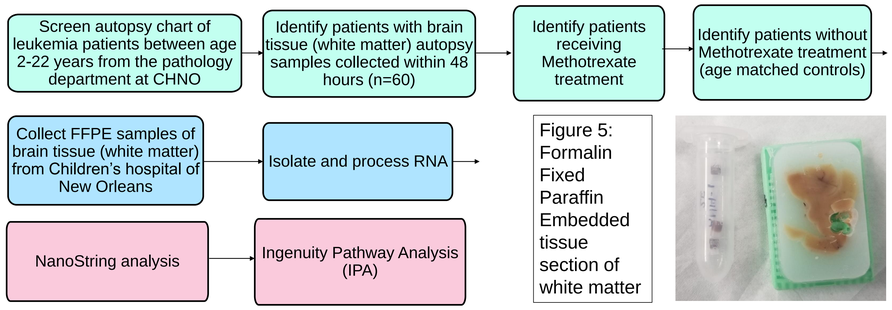

Figure 5: Formalin Fixed Paraffin Embedded tissue section of white matter

Genes with more than 3X over (+) or under (-) expression in white matter of autopsy samples

Genes	Location (chromosome, top/bottom, band, sub band)	Protein	Function	Clinical Significance and Health Risks	Patient vs. Control
GJA1	6q22.31	Connexin 43	Connexins play a role in cell-to-cell communication by forming channels, or gap junctions, between cells. Allow for the transport of nutrients, charged particles (ions), and other small molecules that carry communication signals between cells.	• Nonsyndromic hearing loss • Oculodentodigital dysplasia, a condition characterized by abnormalities of the eyes (leading to vision loss), teeth, and fingers • Coloboma: missing pieces of tissue in structures that form the eye causing vision loss • Critical congenital heart disease	+8.17
HSPB1	7q11.23	Heat shock protein beta-1	Protects cells under adverse conditions such as infection, inflammation, exposure to toxins, elevated temperature, injury, and disease.	Overexpression is associated with Charcot-Marie-Tooth disease: sensory and motor neuropathies that damage peripheral nerves, causing loss of sensation, weakness, and atrophy of muscles in the feet, legs, and hands.	+5.72
AGT	1q42.2	Angiotensinogen	Part of the renin-angiotensin system, which regulates blood pressure and the balance of fluids and salts in the body.	Angiotensin blood vessels constriction, leading to increased blood pressure. Stimulates production of the hormone aldosterone, triggering salt and water absorption by the kidneys.	+5.23
OLFML3	1p13.2	Olfactomedin like 3	Strongly expressed in ocular tissue of human and animal embryos. It plays an angiogenic (blood vessel formation) role in ocular tissue.	Loss of OLFML3 expression suppresses microglia biological functions and leads to Amyotrophic lateral sclerosis (ALS). Also associated with glaucoma due to retinal ganglion cell (RGC) loss from insufficient blood supply.	-4.6
P2RY12+	3q25.1	P2Y purinoceptor 12	During clot formation, the P2RY12 receptor protein helps platelets cluster together to form a clot in order to seal off damaged blood vessels and prevent blood loss.	• Bleeding disorders • Hemorrhagic disease • Thrombocytopenia: Low blood platelet count	-4.82
CD24	6q21	Signal transducer CD24 or Small cell lung carcinoma cluster 4 antigen	CD24 is a cell surface protein that has various and diverse roles in cell adhesion and signaling, B lymphocyte and neuronal development, autoimmune diseases, and cancer.	• Low expression of CD24 leads to cell proliferation and metastasis of breast cancer stem cells. • Damage to the myelin sheath or protective covering that surrounds nerve fibers in the brain, optic nerves and spinal cord.	-8.26

Conclusions

Future directions:

- Continue to test more samples and analyze data
- Perform Ingenuity Pathway Analysis (IPA) to determine other pathways the genes described above are involved in

Limitations:

- Lack of evaluation of genes other than those involved in neuroinflammation

Future research:

- Evaluate genes involved in other pathways in the body to create a more comprehensive understanding of the epigenetic effects of methotrexate on pediatric cancer patients
- Examine other agents or combinations of other treatments
- Examine how these late effects impact survivors into adulthood

Not only will the findings of our study be important to future research, but it will also be essential to physicians who provide late effects care so that they may tailor their treatments to each specific patient, their cancer, its treatment, and the late effects they experience. By creating a better understanding of late effects in pediatric cancer patients, we can help improve the quality of life of childhood cancer survivors.

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