INTERACTION MODEL BETWEEN NATURAL KILLERS CELLS AND LEUKEMIC CELLS: FIRST GOALS AND PRELIMINARY DATA.

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INTRODUCTION:

Acute Leukaemia is the most common cancer in childhood and adolescents. Contemporary chemotherapy protocols achieve 75% overall survival; this results did not improve during the last several years. Indeed, the standard options of conventional chemotherapy and hematopoietic stem cell transplantation are not likely to improve treatment results. A new treatment approach is represented by the anti-tumour immune mediated inherent allo-reactivity of natural killers cells (NKc).

Within innate immune responses, the NKc constitute a small population of circulating lymphocytes. NKc are notable for their ability to spontaneously destroy some tumor cell lines and virally infected populations *in vitro*, without requiring prior immunization or activation. The role and interesting of allo-reactivity of the NKc for the treatment of leukaemia and refractory solid tumours has been increasing in the last few years.

We try to know more about the role of the interaction between NKc and leukemic cells; about this topic, we started a prospective trial in October 2012. The trial's goals were: describing the match or mismatch of NKc receptors (KIR) with leukemic cells, the cytotoxicity and the role of serum cytokines.

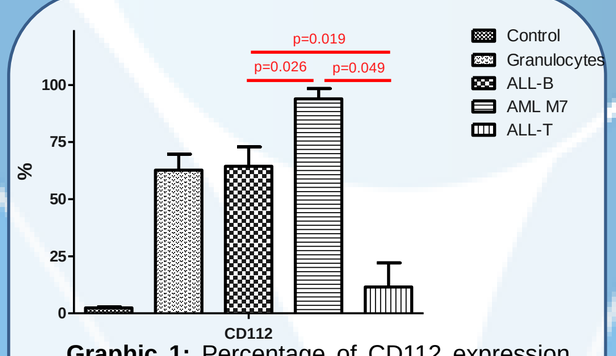
METHODS:

We have included all the new diagnoses of leukaemia, in our hospital from October 2012 till January 2014. In total, we get 20 acute lymphoblastic and myeloblastic leukaemia. One patient was excluded because she refused to sing the consents.

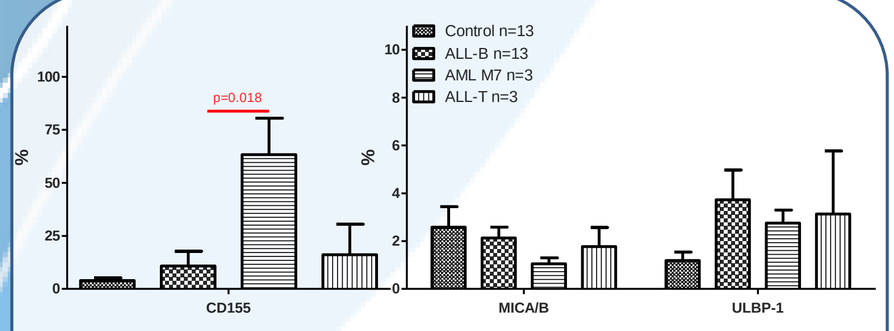
We performed an indirect immuno-flourescence and flow cytometry in all samples to recognize the KIR, HLA and other CD receptors. Sera samples and DNA were stored for a later analysis into the bio bank service, before start the treatment.

A cytotoxicity assay was performed with samples from those patients scheduled to haematopoietic stem cell transplantation and their healthy donors.
A cellular line K562 was used as control, expressing cell lysis percentage by flow cytometry.

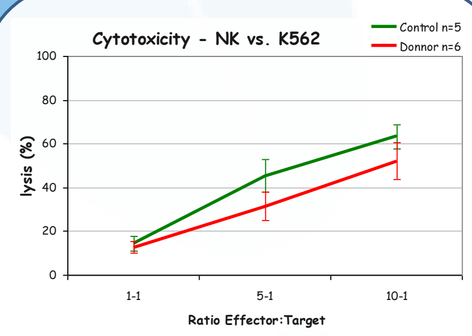
All data were analysed with SPSS for windows and the study has got the approval of the hospital's ethic committee.



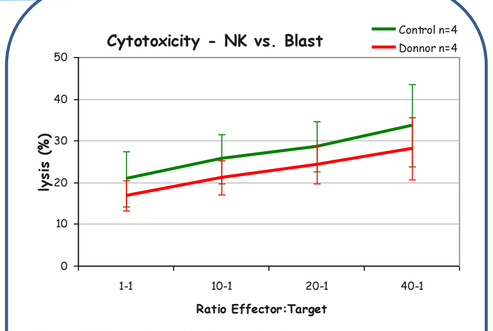
Graphic 1: Percentage of CD112 expression among healthy B lymphocytes of patients, B-ALL, M7 AML and T-ALL. AML blasts showed the highest expression levels and B-ALL blasts expressed more CD112 than T-cell blast.



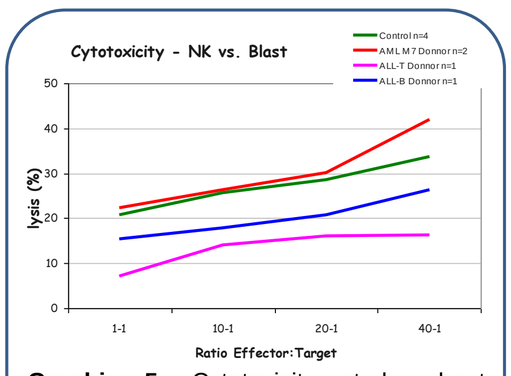
Graphic 2: Percentage of CD 155, MICA/B y ULBP-1 among B lymphocytes of patients, B-ALL, M7 AML and T-ALL. AML blasts showed the highest expression levels of CD155.



Graphic 3: Cytotoxicity study about average percentage of NKc lysis in bone marrow donors and healthy controls against K562. There is any statistical signification.



Graphic 4: Cytotoxicity study about average percentage of NKc lysis in bone marrow donors and healthy controls against leukemic blast. There is any statistical signification.



Graphic 5: Cytotoxicity study about average percentage of NKc lysis in bone marrow donors and healthy controls against leukemic blast, split in types of leukaemia. No statistical signification.

RESULTS:

The sample was: three children with ALM M7, thirteen B-ALL and three T- ALL. We collected data from 13 boys and 6 girls; the median age was 6 years (age range from 1 to 13). Six patients underwent hematopoietic stem cell transplant, watching disparities around 0 to 8 in KIR receptors. The Bx genotype was the most frequent NKc genotype in relapsed patients. The estimated rate of survival was 78,9% and a 10,5% of mortality, with a short follow up period between 3 to 14 months.

Blast cell has got a mayor expression of CD155 and CD112. Similarly, leukemic blast cells and granulocytes expressed significantly higher levels of CD112 than B cells.

We found no differences among stem cell donors and controls and the percentages of lysis in the cytotoxicity assay, no differences among the different leukaemia subtypes. However, the small sample size precludes extracting firm conclusions.

CONCLUSIONS:

Some studies reported the over expression of the CD155 gene in human tumours like myeloid leukaemia and neuroblastoma. The pathway is not known yet, but CD155's over expression might induce a down-regulation of the NKc immune response. This could partially explain the poor outcome of theses patients. Indeed, in our study we have found an over expression of CD155 and CD112 in leukemic blast cells which was particularly prominent in AML patients. Sera sample analyses for CD155 and other soluble factors are currently underway.

The next steps in the study will be: testing the cytotoxicity of patients at the end of treatments and link these results with KIR and HLA genotype. Nowadays we found no differences in outcome attending to HLA genotype, NKc genotype and KIR expression. Furthermore, no differences of cytotoxicity studies were found between the leukaemia subtypes. These results might be explained by the small sample size.

This project is in an early stages, and we need to collect more samples, analyses the sera samples and make more cytotoxicity tests. Ours aims are exploring about: the genomic profile of NK cell receptors and their ligands, the surface expression of receptor on NKc and T lymphocytes, the role of plasma soluble factor influencing NK-cell function in tumour response and outcome, and the *in vitro* cytotoxic activity of autologous and allogeneic NK cells against leukemic cells. Our hopes are helping in the immunotherapy progress and improving the global survival of children. The goals of the immunotherapy in cancer are under constructions.

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