The utility of Borrelia-specific T cells as diagnostic biomarkers of Lyme disease

Liselotte Brix[1], Amir Ameri[1], Dilek Inekci[1], Yngvar Tveten[2], Kivin Jacobsen[1], Stig Andersen[1], Anna Viktoria Vaaben[1], Sambor Grygorczuk[3] and Dag Nyman[4]
[1]Immudex, Copenhagen, Denmark; [2]Sykehuset Telemark, Skien, Norway; [3]Medical University in Bialystok, Bialystok, Poland; [4]Bimelix, Mariehamn, Åland

1. BETTER DIAGNOSTICS FOR LYME DISEASE

Diagnosis of Lyme disease (LD) is still a challenge due to lack of predictive biomarkers. Most of the currently available diagnostic laboratory tests are based on serological detection of antibody responses against Borrelia-specific antigens (1). The major limitations of these test are their lack of ability to provide an early diagnosis with a high sensitivity and specificity and distinguish between active and inactive infection. Hence, the development of a novel diagnostic biomarker assay that can provide an early and accurate diagnosis and distinguish between active and past cleared disease is an urgent need (2).

To improve the value of the current Borrelia diagnostic landscape, we are investigating the potential of using measurement of Borrelia-specific T cells as a diagnostic relevant biomarker.

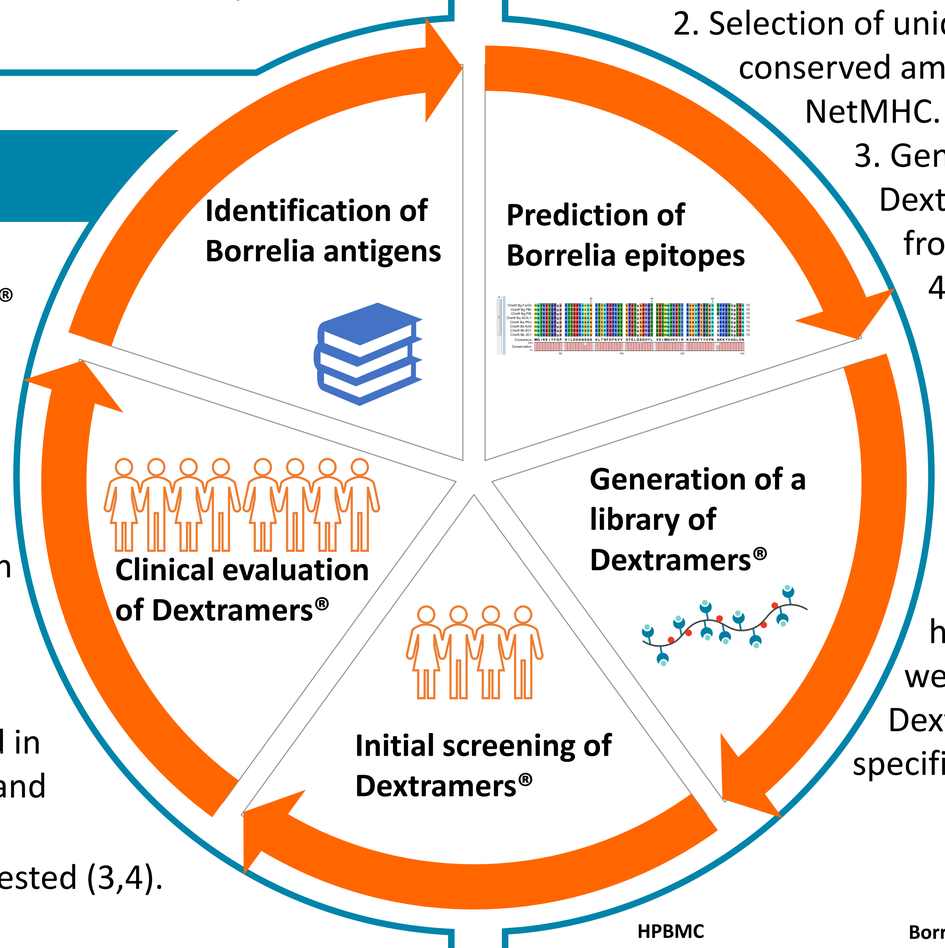
2. BORRELIA –SPECIFIC DEXTRAMER® ASSAY

To detect Borrelia-specific T cells, we have developed a Borrelia-specific MHC Dextramer® assay. The MHC Dextramer® reagents consists of a dextran polymer backbone carrying an optimized number of fluorochromes and MHC molecules displaying Borrelia-specific T cell epitopes of interest. This technology allows direct measurement of Borrelia-specific T cells in the blood with a high avidity for the specific T cell. Borrelia-specific Dextramers panels were generated using a 4 step process:

1. Literature-based identification of 40 potentially immunogenic Borrelia proteins.
2. Selection of unique and HLA-A*0201-specific epitopes conserved among Borrelia strains using the prediction tool NetMHC.
3. Generation of a library of 253 Borrelia-specific Dextramers® and screening in panels on samples from Borrelia patients and healthy controls.
4. Individual screening of the 40 best performing Dextramers® to verify their identification of Borrelia-specific T cells.

Results

11 of the Dextramers tested in step 4 identified Borrelia-specific T cells in 25-100% of the patient samples and showed no response in healthy control samples. The 11 Dextramers were selected for the development of a Borrelia Dextramer® assay for the measurement of Borrelia-specific CD8+ T cells in blood by using flow cytometry.



3. CLINICAL EVALUATION

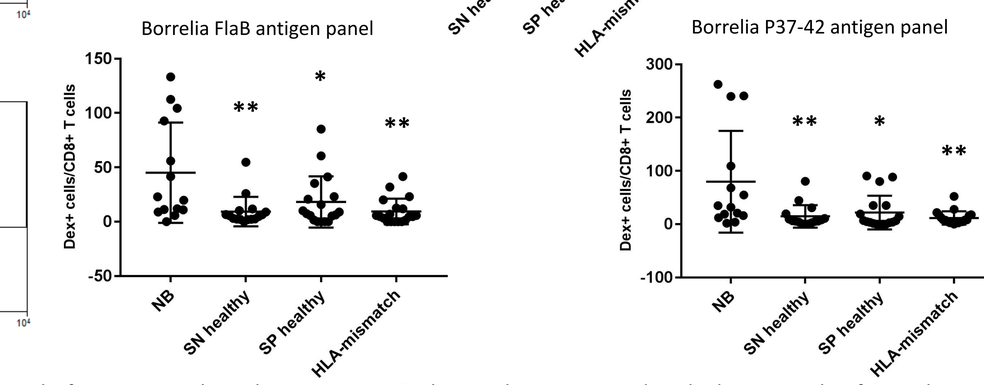
Study set-up

The diagnostic value of the Borrelia Dextramer® assay was evaluated in a small retrospective study of 14 neuroborreliosis patients, 16 healthy seronegative control subjects, 18 healthy seropositive controls and 19 HLA-mismatched controls. The 11 Borrelia Dextramers® were tested in 3 panels along with a negative control panel and a positive control panel. All samples were obtained from an in-house biobank of 400+ blood samples from Borrelia patients and healthy controls collected in three endemic areas in Åland, Norway and Poland and in Denmark over a period of 3 years.

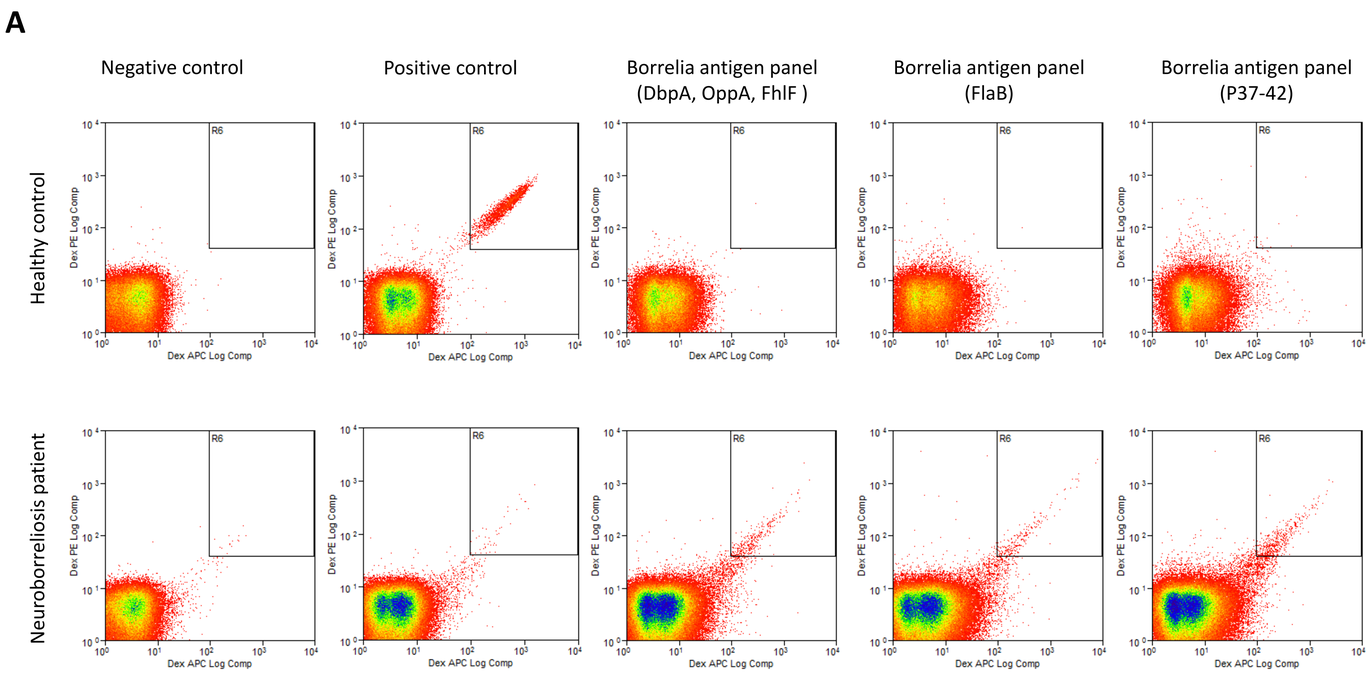
All samples were HLA-typed and serologically tested (3,4).

Flow cytometry analysis

1. Prepare lymphocytes
2. Viability staining of cells
3. Stain cells with Dextramer® assay
4. Stain cells with anti-CD3-PE-CY7, anti-CD14-FITC and anti-CD8-PB
5. Wash cells and analyze by flow cytometry
6. Exclude dead cells, monocytes and $CD8^-$ cells
7. Identify Borrelia-specific $CD8^+$ T cells



4. THE DEXTRAMER® ASSAY IDENTIFIES BORRELIA-SPECIFIC T CELLS



Results of clinical evaluation. A) Flow cytometry results from Borrelia Dextramer Assay analysis of a healthy control sample and sample from a neuroborreliosis patient. Each samples was tested with three panels of Borrelia-specific Dextramers and positive and negative control Dextramer panels. **B)** Overall results of all samples tested shown as the Borrelia-specific T cell response measured in samples from neuroborreliosis compared to response measured in seronegative, seropositive and HLA-mismatched samples from healthy control subjects. The healthy seropositive control group include subjects with a past cleared borrelia infection as well as forest workers continuously exposed to ticks. *p<0,05, **p<0.01. NB: neuroborreliosis, SN: seronegative, SP: seropositive.

CONCLUSION

The present findings show that the novel Borrelia Dextramer® assay is a promising tool to identify Borrelia-specific T-cell responses in NB patients. The Borrelia-specific T-cell response is significantly elevated in NB patients compared to healthy controls. Our results indicate that this test has potential to be able to discriminate between active and past cleared infection.

REFERENCES

1) Aguero-Rosenfeld et al. Diagnosis of Lyme Borreliosis. Clin Microbiol Reviews 2005 18(3):484–509
2) Marques et al. Laboratory Diagnosis of Lyme Disease Advances and Challenges. Infect Dis Clin of North America 2015 29(2): 295-307
3) Stanek et al. Lyme Borreliosis: Clinical case definitions for diagnosis and management in Europe. Clin. Micro. Infect. 2011 17(1):69-79
4) Mygland et al. EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. Eur J Neurol. 2010 17(1):8-16

This work has been performed in a Eurostar's funded consortium comprising Bimelix, Åland; Telemark Sykehus; Norway, Bialystok Hospital, Poland and Immudex, Denmark.

eurostars™

immudex
PRECISION IMMUNE MONITORING