Circulating lymphocyte subsets as prognostic markers in multiple sclerosis

IMPACT PARAGRAPH

1. What is the main goal of the research reported in the thesis and what are the most important results and conclusion?

Over the last few decades, the clinical care of multiple sclerosis (MS) patients has evolved dramatically. An influx of new disease modifying treatments, as well as the introduction of brain imaging through magnetic resonance imaging (MRI), has provided the clinician with many more options to monitor and treat MS patients. Despite these advancements, much remains unknown or suboptimal in our knowledge of MS. One of these things is the lack of a prospective biomarker for disease activity in MS. Currently, no curative treatment for MS exists, and as such patients are committed to lifelong use of immune modulating therapies to suppress further disease activity. Currently, the choice to intensify treatment is based on clinical relapse or MRI activity, which are both retrospective measurements, *i.e.,* the choice to intensify treatment can only come after new activity is recorded. Thus, damage to the CNS has already occurred and may have caused permanent disability in the MS patient. Identification of predictive markers may help make choices regarding treatment before disease activity occurs, which could prevent or delay disability progression.

To find such a marker, it is tempting to look at the cells which effectuate the damage in MS. Classically, MS was considered to be caused mainly by aberrant T-cells. However, more recent insights have shown roles for other lymphocytes, such as B cells and NK cells, in the MS disease process. Despite these new insights, very little is known about the significance of the presence of these lymphocytes in the serum of MS patients. In other words, while we better understand which cells contribute in which manner to the MS disease process, we still do not know what the relative presence of these cells in the blood means for MS patients. If the inflammatory process of MS is mediated by these lymphocytes, then early alterations in the composition of lymphocytes may show prognostic value for disease activity.

The main goal of this research was to explore prognostic value of the lymphocyte composition in the blood of MS patients, with a focus on the lymphocyte subsets which gained recent attention as key players in the disease process. As we performed this research in a cohort receiving high-dose vitamin D_3 supplementation, we also aimed to further elucidate the effects of vitamin D_3 in MS.

Starting with vitamin D, we report no association between vitamin D levels and serum levels of the neurofilament light chain biomarker for axonal loss, implying this biomarker

can be used in our cohort without the need for correction for vitamin D supplements. Second, we report that certain genetic subgroups in our cohort had different serological responses to high-dose vitamin D_3 supplementation, with some showing a lower increase in serum vitamin D than others. This has implications for present and future vitamin D_3 supplementation studies, as it implies that genetic subgroups of patients may benefit differently from supplements. It may be true that only a specific subgroup benefits from vitamin D_3 supplementation, which may explain the varying results from earlier vitamin D_3 supplementation studies and would aid MS therapy by supplementing vitamin D_3 to specific MS patients.

Moving on our lymphocyte related results, we report a prognostic value of a natural killer (NK) / T cell ratio for MS disease activity. Not only does this finding underline the interaction between NK cells and T cells in MS, it also proposes a new and accessible biomarker for MS disease activity. Then, we go on to show that this NK/T cell interaction is partially mediated through the IL-2 – IL-2 receptor pathway. This finding adds to the understanding of the NK/T cell interaction in MS.

To continue, we report a prognostic value of transitional B cells for MS disease activity. Again, this finding both shows the potential protective role of transitional B cells in MS, as well as providing another potential new and accessible prognostic marker for MS disease activity. Additionally, we find that a persistence of the elevated anti-EBNA-1 titre in serum is associated with an increased risk of MS disease activity.

Finally, taking these mentioned prognostic markers from our results, we construct a rudimentary prognostic model, showing fact that MS patients without disease activity that have unfavourable levels of one prognostic marker, appear to compensate this by having favourable levels of another marker. By contrast, patients with unfavourable levels of multiple prognostic markers show a higher risk of MS disease activity. Here, we propose that by finding more involved prognostic markers, one may construct a predictive model involving markers in the circulation that indicates a risk of MS disease activity in the next year. We conclude that certain lymphocytes show prognostic value for disease activity in MS patients, and that by searching for additional markers we may not only further our understanding of the MS disease process, but also add to the prognostic model in order to more accurately determine risk of disease activity.

2. What is the potential contribution of the results from this research to the scientific community, and if applicable to public sectors and public challenges?

Our vitamin D related research contributes to later high-dose vitamin D trails, especially our results concerning the difference in serological response between genetic subgroups. Considering high-dose vitamin D_3 supplementation reduces MRI lesions, as shown in the SOLAR study, this finding could imply that certain patients have a better response to supplementation than others based on their genetics. Later vitamin D supplementation studies should include these genetic variations in order to further establish the exact effects of these genetic variations, which may ultimately point towards a genetic subgroup of patients that benefits most from high-dose vitamin D_3 supplementation.

Generally, our results regarding the prognostic value of lymphocytes may be used in two ways. First, we describe prognostic values for several cell subsets for disease activity in MS. These cell subsets may either show an association with an increased or a decreased risk of MS disease activity. Although this implicitly implies their respective detrimental or protective roles, it remains unclear how these roles are carried out *in vivo*. As such, our results may prompt researchers to further investigate our reported cell subsets to find their mechanism of action in MS disease activity, ultimately aiming to uncover new therapeutic targets for MS treatment. The second way our work contributes to the scientific field is by laying the foundation for a predictive model of MS disease activity using serological markers. While our model at the end of this thesis is quite preliminary, it does illustrate the potential of serological markers as predictors of disease activity. As such, other groups may use this knowledge to find more circulating markers, or to extend and/or refine our currently proposed model.

3. For whom may the results of this research be interesting and/or relevant? And why?

Our results may be of interest to a multitude of groups thanks to the broad implications of our research.

First, clinical researchers should find our results interesting for several reasons. We report a genetic impact in our cohort receiving high-dose vitamin D_3 supplementation, which implicates that these genetic factors should be considered/corrected for in further vitamin D studies. Additionally, our findings regarding the prognostic value of several lymphocyte subsets should prompt researchers to include these immunological parameters in later prognostic studies. Furthermore, the results of **Chapter 7** should interest clinical researchers investigating systems biology, as it lays a foundation for immune profiling in MS patients.

Laboratory researchers should be interested in our efforts to further elucidate the NK – T cell interaction, especially through the IL-2 – IL-2 receptor pathway. Additionally, as we show that patients with a unfavourable NK/T cell ratio may compensate with a favourable level of transitional B cells, the interaction of B cells with NK cells and T cells (The NK – T- B cell interaction) is shown to be an interesting and relevant topic and may prompt researchers to elucidate it further.

Next, our results are also of interest to clinicians, as they provide further insights into the workings of a commonly prescribed dietary supplement in MS patients (*i.e.* vitamin D). Additionally, clinicians should be excited for the prospect of a possible prognostic/predictive tool for MS disease activity. Although this tool is at the very early stages, if it indeed reaches clinical practise, then it is easy to imagine the significant impact it would have on clinical decision making.

Finally, MS patients may be interested in the developments surrounding vitamin D supplementation, especially the implication that some MS patients may show genetic predisposition to a stronger response to vitamin D supplementation, which may in turn show clinical benefit, although this remains to be investigated. Additionally, we provide insights in what the composition of the immune system in blood samples means for the patient and how we may use it to treat their chronic disease more effectively.

4. In what way could these target audiences be involved in and informed of the results of this research, so that the gained insights can be used in the future?

First and foremost, all our research described in **Chapter 2** through **Chapter 7** has been published in established peer-reviewed scientific journals and is thus readily accessible to the scientific community at a trustworthy source. Additionally, most of our chapters have been presented at national or international conventions, even though due to the COVID-19 pandemic, the number of presentations has been limited. Regardless, other renowned MS research teams have submitted proposals to further investigate NK cells in multiple sclerosis, which may be partially inspired by our published and/or presented results. Finally, a summary of some chapters has been published on the website of the Dutch national MS foundation (Nationaal MS fonds), which makes our results accessible at patient level for people with MS.