**Identifying Immunological and Movement Pattern Biomarkers for the Prediction of Transition from Acute to Chronic Low Back Pain**

**Abstract**

Chronic low back pain (CLBP) is a complex disease that causes disability, functional decline, and reduced quality of life and leads to higher medical and non-medical-related costs for patients, employers, and healthcare providers. CLBP is usually defined as pain that lasts more than three months. **Establishing a predictive model for the transition from acute to CLBP may have significant personal, social, and economic benefits, enabling clinicians to accurately predict which patients are at high risk of developing CLBP still in the early stages of the condition.** Prognostic models that consider patient characteristics may provide evidence-based input for treatment decisions.

In the proposed study, we will use sophisticated technology to examine major new aspects related to CLBP: the immune system status and movement behavior pattern. Inflammatory processes play a central role in the pathogenesis of LBP as pro-inflammatory cytokines are elevated in painful conditions and may promote pain. Physical activity is known to have positive effect on the immune system function, thus mediating pain level, and may also affect the LBP chronicity process.

We will examine these aspects at a number of time points over a period of three months and compare patients who eventually develop CLBP with those who do not, thus enabling us to determine the earliest time point of the transition toward chronicity.

**The overall hypothesis is that immunological and movement pattern biomarkers may help us to understand the transition from acute to chronic low back pain and allow prediction of those patients who will develop CLBP.**

We will examine patients with an acute episode of LBP and perform baseline examination which will include questionnaires regarding pain and function (fear avoidance, depression, physical activity level, type of work, LBP severity), physical examination (functional sit-to-stand test, spine range of motion), and a blood test. Each participant will receive an accelerometer (Wireless ActiGraph GT3X) to wear continuously for a period of 3 months in order to monitor his/her movement pattern and physical activity behavior. Participants will rate their perceived pain, fatigue, and movement limitations using a mobile application.

Examination of immune system profile and activation will be performed using a new state-of-the-art methodology called mass cytometry (commercially called “CyTOF”) that enables high-resolution monitoring of an individual’s immune system. CyTOF is a single cell proteomics antibody-based technology allowing to profile ~40 proteins per cell.

Blood tests and physical examination will be carried out at 0, 2, 4, 8, and 12 weeks. A participant will be defined as CLBP or Control if he/she still has or does not have pain after 3 months, and a comparison between groups will be performed for all measurements.

**Expected significance:** A predictivemodel for the transition from acute to chronic LBP is essential in understanding LBP, and will help to improve treatment and decrease health system costs.

**Research program**

1. **Scientific Background**

Chronic low back pain (CLBP) is a complex disease with highly heterogeneous symptoms, level of pain, function, and more, making it difficult to decide on appropriate treatment. In addition, the extent to which a patient is at risk of developing CLBP is essential in making treatment decisions.

Establishing a predictive model for the transition from acute to CLBP could have significant personal, social, and economic benefits, enabling clinicians to accurately predict who is at an elevated risk of developing CLBP while still in the early stages of the condition. Prognostic models that consider patient characteristics may aid in providing evidence-based input for treatment decisions.

***Low back pain prevalence***

Low back pain (LBP) is the most common disorder causing disability, functional decline, and reduced quality of life. LBP leads to increased medical and non-medical-related costs for patients, employers, and healthcare providers. In the United States, treatment of LBP and related spine disorders represents the most expensive medical problem, with nearly twice the burden of any other health condition.1 The national cost of back pain in 2015 in Europe ranged from $259 million ($29.1 per capita) in Sweden to $71.6 billion ($868.4 per capita) in Germany.2

The probability that symptoms of LBP will appear during one's lifetime is 80-85%.3–5 The majority of low back problems (90%) are considered non-specific,6,7 while the remaining 10% of patients are specifically diagnosed with malignancy, radiculopathy, stress fractures, spinal stenosis, spondylolisthesis, or blood vessel problems.

***Low back pain and chronicity***

While most patients with nonspecific LBP will be pain-free within 6 weeks,8–10 the estimated prevalence of developing CLBP is between 4-40%.1,9 Chronic pain is defined as pain that is not relieved within an expected time frame, does not respond to acceptable analgesic treatment, and in general, lasts more than three months.11,12

The transition from acute to chronic low back pain has been the focus of many studies attempting to identify risk factors for the chronicity process, with the aim of hampering this transition. The biopsychosocial (BPS) model of LBP, proposed by Engel in 1977,13 acknowledges not only biological but also psychological and social influences on pain and promotes a more humanistic perspective of healthcare. It was demonstrated that psychological (behaviors, beliefs, distress, depression, anxiety, and fear) and social factors (financial, family, and work-related issues) can be associated with the improvement of a patient’s symptoms, and be related to the persistence of pain and disability.13 Among the many risk factors that were suggested to affect the prevalence of nonspecific CLBP are demographic parameters, low physical activity, occupational factors, perceived higher pain intensity, higher body weight, and psychological factors such fear-avoidance belief, stress, anxiety, and depression.14,15

Although the risk factors are known, there is still a lack of knowledge regarding the transition process to chronicity which would enable early preventive and therapeutic intervention.

***Immune system and pain***

Previous evidence shows that inflammatory processes play a central role in the pathogenesis of LBP.5,16 Inflammatory response has an important role in pain by sensitizing nociceptor neurons, mainly through the production of inflammatory cytokines.17

Cytokines such as interleukin 6 (IL-6), IL-8, and tumor necrosis factor (TNF) were found to contribute to the activation of nociceptors that increase pain hypersensitivity, and their levels in the plasma are associated with a higher perception of pain.18–20 Li *et al.*21 described alteration of both pro- and anti-inflammatory cytokines (IL-6, IL-10) and suggested that an imbalance between pro-inflammatory and anti-inflammatory mediators contributes to the pathophysiology of CLBP. The levels of pro-cytokines are also recognized for their muscle catabolic effect, which may explain disability.6,22–24 In contrast, other anti-inflammatory markers, including IL-4 and IL-10, have been observed to be negatively correlated with pain severity in LBP.

The inflammatory profiles of patients with acute and chronic LBP are distinct. In a recent study that characterized and compared the inflammatory profile of acute and chronic LBP patients, the production of pro-inflammatory cytokines TNF alpha, IL-6, and IL-1 beta was found to be elevated and the anti-inflammatory cytokine IL-10 was reduced in both LBP patients.25 The differences between acute and chronic phases of LBP include a higher production of TNF alpha, IL-1 RA, and sTNFR2 in CLBP, while during the acute phase, a higher level of IL-2 interferon gamma was observed.25 These findings require further investigation in order to understand the transition from acute to chronic LBP.

Blocking pro-inflammation pathways with anti-inflammatory drugs is routinely used to manage pain and inflammation for musculoskeletal pain.26 Understanding the role of anti-inflammatory cytokines in the analgesic effect will further improve response to treatment and prevent the chronicity process.20,27

Technological developments in recent years allow broad high-resolution profiling of the immune system, something which has not been possible until now. These developments include the possibility of simultaneously measuring hundreds of different immune component (cell types, cytokines, mRNA, and others).28 This new technology allows a deeper understanding of how the different features are related to each other, and overcomes the great variance observed when measuring only a few representative components (e.g., C-reactive protein and IL-6).29 This may lead to the identification of comprehensive targets for the prediction of clinically relevant information. More recently, novel fluorophore and laser systems are driving the discovery of new immune cell subclasses as well as important functional states.30 This may aid in predicting an individual’s disease or condition early or in guiding treatment. For instance, immune-based blood biomarkers have been identified which can help predict hip surgery recovery time and pain responses.31

We believe that a more extensive immune profile that allows for assessment of the complexity of the immune system, in a more comprehensive way, would yield better information about the role of the immune system in the transition from acute to CLBP.

***Physical activity and movement pattern***

Changes in motor behavior and movement patterns following pain and injury occur initially to protect the damaged tissue and to reduce the actual or anticipated threats. However, these guarded behaviors are thought to sub-optimally load tissues over time, leading to pain, re-injury, and disability.32

Understanding how physical behavior changes following injury is a complex task since motor behavior and movement patterns are affected by many factors (such as pain, injured structure, acquired behavior, etc.). One of the leading theories explaining physical activity reduction in LBP is based on avoidance-persistence behaviors.33 The physical activity of patients with LBP has been studied extensively, but the majority of studies were performed using self-reported questionnaires,34–36 which are insufficiently valid for measuring the intensity of physical activity and movement pattern during the day. As far as we know, only a few studies37,38 have continuously measured physical activity by tri-axial accelerometer for 1 week during the admission phase for CLBP. Collecting movement data by accelerometer allows objective monitoring and evaluation of the participant's movement pattern.39,40

One of the key recommendations in acute and chronic LBP treatment guidelines is to stay active and conduct exercise therapy. Regular exercise was demonstrated to be an effective treatment for low back pain resulting in reduced disability and pain severity.41,42 One explanation of the underlying mechanisms for exercise-induced pain relief is that physical activity is associated with reducing systemic inflammation.43

Although physical activity has been shown to improve pain and function among individuals with acute or chronic LBP pain, movement pattern during the transition from acute to chronic LBP and the effect these movement patterns have on transition require further investigation.

***Physical activity and the immune system***

In general, repeated moderate-intensity exercise enhances the immune function response, reinforces antioxidative capacity, and reduces oxidative stress, leading to reduction of the incidence of inflammatory diseases and conditions.44–46

Activation of the immune system is a response to a stressor such as exercise, aiming to restore cellular homeostasis. The immune system is very responsive to exercise,47 and acute and chronic physical exercise significantly alters the immune system.48 The inflammatory process plays a crucial role in the homeostasis, mainly through active defense against various harmful stimuli such as neurotropic viral infections and/or traumatic damage, promoting the re-establishment of cellular and tissue function.46

Exercise training has been reported to counter inflammation elements of some disease processes49 characterized in part by high inflammation, oxidative stress, and immune dysfunction, by stimulating many cellular and molecular changes throughout body tissues that promote anti-inflammatory and antioxidant responses, and augmenting immunosurveillance.47

An individual’s mobility behavior can influence the immune system profile50 which mediates pain level, thus affecting the LBP chronicity process.

1. **Research Objectives & Expected Significance**

CLBP is considered to be a disease in its own right. It is one of the major causes of comorbidity, other related symptoms, disability, and poor quality of life, with no optimal treatment available. As such, it is essential to identify additional risk factors and employ novel technologies in order to discover factors that may affect or predict the transition from acute to chronic low back pain. For this, longitudinal studies, beginning at low back pain onset, are essential to understand the pain chronicity process. An enhanced understanding of the risk factors for chronicity in low back pain is essential for providing evidence-based input for treatment decisions.

In the proposed study, we will use sophisticated technology to examine new major aspects related to immune system status and movement behavior and their contribution in predicting the transition from acute to CLBP. We will examine these aspects at several time points over a period of three months and compare patients who eventually develop CLBP with those who do not, thus enabling us to determine the earliest time point of the transition toward chronicity and to develop a model to predict LBP chronicity.

**Objectives:**

The overall goal of this study is to identify immunological and movement pattern biomarkers for the prediction of transition from acute to chronic low back pain.

**Specific aims:**

* + - 1. To characterize immunological profile, movement pattern, demographic characteristics, functional ability, pain perception, and psychosocial traits of individuals who develop CLBP with those who do not.
			2. To define and cluster the changes in immunological profile during three months of follow-up from the acute event.
			3. To define and characterize changes in movement patterns during three months of follow-up from the acute event.
			4. To examine the changes in functional ability, pain perception and psychological traits during three months of follow-up from the acute event.
			5. To identify the time of transition from acute to CLBP from immune profile and movement pattern.
			6. To develop a predictive model to identify patients in their acute phase who are at risk of developing CLBP.

**Expected significance:** Immune system and movement pattern are key elements in pain and function. This proposal will lay the groundwork for understanding the role of the immune system network profile and movement pattern in the transition from acute to CLBP.

Examining these factors, together with known aspects of LBP, will enable deeper understanding of CLBP. Mapping this relationship will improve clinical decision-making and open the door to achieving a personalized and effective therapeutic plan to further improve a patient's symptoms and function. The study will have long-term utility as improving personalized treatment can decrease the burden on the health system and reduce the costs of treatments.

1. **Detailed Description of the Proposed Research**

**Working hypothesis:**

* + - 1. Differences in demographic, movement pattern, functional, pain perception, psychosocial, and immune cluster parameters at all time points will be found between patients who develop CLBP and those who do not.
			2. Subjects who will develop CLBP will demonstrate higher pro-inflammatory status in immune system, already after one month and during three months of follow-up.
			3. Subjects who will develop CLBP will demonstrate lower physical activity, higher medial-lateral (y-axis amplitude) movement, greater movement during sleep already after one month and during three months of follow-up.
			4. Subjects who will develop CLBP will demonstrate lower functional score, greater pain perception, greater fear from movement after one month, and during three months of follow-up from the acute event.
			5. Transition from acute to CLBP will be demonstrated via the immune system and movement pattern, already 2 weeks after acute event.
			6. It will be possible to establish a predictive model for the transition from acute to CLBP from immune system and movement pattern variables.

**Research design & methods:**

We chose to study LBP as it is one of the major musculoskeletal disorders, with accompanying high health system costs, and remains a challenge for physicians to treat, having no specific solution. Developing and examining a new model of LBP chronicity will provide further insight into CLBP pathophysiology and may lead to the identification of novel targets for treating symptomatic CLBP patients, leading to personalized treatment. We believe that studying new aspects of biology-immune system responses and movement pattern changes along the chronicity path will allow better practical intervention and wiser resource distribution to reduce the incidence of CLBP.

**Research plan:**

For this prospective study, individuals seeing a health practitioner for a first episode of acute low back pain or recurrent LBP after a pain-free period of at least 12 months will be recruited.

The participant will be followed longitudinally for a period of three months. Following completion of the study period, participants will be divided into two groups: (1) those who develop CLBP (i.e., CLBP group); and (2) those who do not (i.e., Control group).

The research plan will be submitted for approval of the Helsinki committee in Maccabi Healthcare Services.

A detailed explanation of the study design and all tests (see Methods section) will be provided to all participants at the beginning of the study and written informed consent will be obtained.

***Participants***

This prospective study will include individuals with acute episodes of low back pain.

An *a priori* power analysis was conducted using G\*Power software51 to determine the minimum sample size required to test the study hypothesis. Results indicated that a sample size of 88 was required to achieve 80% power for detecting a 0.4 effect size, at a significance criterion of α = .05.We will thus use a sample size of 100 (+20 for the exploratory phase, as described below) to test our study hypothesis.52

In order to participate in the present proposed study, subjects will need to meet the following inclusion criteria: age 20-65 years; main complaint of acute nonspecific LBP for less than a two-week period, with a pain score of at least 3 out of 10 on the visual analog scale (VAS); and ability to understand the purpose and instructions of the study.

Subjects will be excluded from participation if LBP is specific (tumor, ankylosing spondylitis, fracture, cauda equina syndrome); two or more of the following signs are present on physical examination: lower extremity weakness in a myotome distribution, decreased sensation in a dermatomal distribution, altered lower extremity deep tendon reflexes, pathological reflexes, a positive straight leg raise (SLR) test, crossed SLR or femoral nerve stretch test; symptoms began immediately after a significant trauma (motor vehicle accident, fall from a height); physical therapy or chiropractic treatment for LBP was provided during the 6 months prior to participation in the study or the subject is currently being treated.

***Research procedure***

The study procedure is described in Figure 1. Participants will be recruited to the study and will undergo a Baseline examination (T0) as soon as possible following onset of their low back pain episode and no more than 2 weeks after. Recruitment will be at the healthcare services clinic by a physician, nurse, research assistant, or through advertisement in healthcare clinics.

Baseline examination will include filling out a questionnaire regarding pain and function (fear avoidance, depression, physical activity level, type of work, and LBP severity) and physical examination to assess functional ability with and without an accelerometer. A blood test will be taken for immune system measurements.

Following this, each participant will receive an accelerometer (Wireless ActiGraph GT3X) and will be asked to wear it on the waist during the day, and on the wrist during the night (when sleeping). In general, the accelerometer will be worn at all times for a period of 3 months in order to monitor movement pattern and physical activity behavior. Participants will be asked to rate their perceived pain, fatigue, and movement limitations on a visual analog scale (grades 1‑10) every day for the first 2 weeks, using a mobile application. During this 2-week period, the researcher will conduct weekly phone calls with participants to confirm their compliance with the study requirements and tasks.

Blood tests and physical examination will be carried out at 0, 2, 4, 8, and 12 weeks at a healthcare clinic.

A participant will be defined as CLBP or non-CLBP if he/she still has or does not have pain after 3 months.

**Figure 1: Outline of Research Design**



***Measurements***

Blood test for immune profile

The immune system profile analysis will comprise two phases: an exploratory phase (Fig. 1) will include collection of blood samples at five time points (T0-acute phase on admission for physician help; T1-2 weeks after T0, T2-4 weeks after T0; T3-8 weeks after T0, and T4-12 weeks after T0) to analyze changes in a very wide immune profile during the transfer process from acute to chronic LBP. For this first phase, the first 20 participants (10 with CLBP and 10 without CLBP) will be monitored and a full analysis of all measurements (five time points and all related variables) will be performed. This will allow us to detect the time points which are most likely to be important for research on the chronicity trajectory. The second phase is aimed at prediction analysis and will examine the three most significant time points selected and variables as detected in the exploratory phase in a larger sample size (100 participants). All blood samples will be stored at -20°C and will be analyzed only after 10 participants from each group (CLBP/control) will be identified after 3 months. According to data analysis of the first 10 participants from each group, samples from the time points indicating a greater alteration of the immune profile will be further analyzed.

Examination of immune system profile and activation will be performed using a new state-of-the-art methodology called mass cytometry (commercially called “CyTOF”) that enables high-resolution monitoring of an individual’s immune system.28,53 CyTOF is a single-cell proteomics antibody-based technology allowing profiling of ~40 proteins per cell (see Preliminary Results). Mass cytometry allows for many molecules to be used in combination to assay a single sample (blood or single cell suspension of tissues).28 We will use an antibody panel designed to provide a high-dimensional snapshot of an individual's immune system. In addition, we will collect peripheral blood for whole blood gene expression data.

Blood (5 ml) will be drawn from subjects by a nurse into sodium heparin tubes and transferred into Prot1 ™ proteomic stabilizer (SmartTube™ Inc.) tubes at a ratio of 1:1.4 and incubated at room temperature for 10 minutes. Tubes will then be stored at -80°C until used for CyTOF. The SmartTube™ system dramatically reduces technical variation by enabling storage of whole blood samples for long periods of time while preserving surface and intracellular epitopes.54 Blood (2 ml) will be drawn directly into PaxGene RNA stabilizer tubes, which will be stored at
-80°C until processing. RNA will be extracted using a PAXgene Blood RNA Kit (Qiagen) and gene expression will be analyzed.

Daily physical activity and movement patterns

Daily physical activity (PA) will be recorded continuously using a tri-axial accelerometer, ActiGraph wGT3X-BT (ActiGraph, Pensacola, FL, USA), with a sampling frequency of 100 Hz. The ActiGraph device is small, 3.5x3.5 × 1 cm, and weighs 14 g. Participants will be asked to wear the ActiGraph wGT3X-BT sensor on the waist during waking hours and on the wrist at night, while sleeping. The following data will be collected: raw acceleration (the physical movements of each patient in 3-dimensional space at 0.1 s intervals), activity intensity, steps, total movement, total sleep time (TST), sleep efficiency, wake after sleep onset (WASO), and sleep fragmentation. The data will be analyzed to show ongoing behavioral patterns (behavior over time), as will cumulative data including the amount of time sitting, standing, walking, and lying down, walking duration, and number of walking episodes.

Initialization of the ActiGraph for recording and downloading data will be done using the manufacturer’s program (ActiLife).

The collected data will be analyzed (abstracted and integrated) and used to determine the individual type and level of physical activity as well as sleep patterns, in addition to identifying groups of patients with similar characteristics. We hope that these data will allow us to distinguish between patients with acute and chronic low back pain (using unsupervised machine learning techniques – clustering). The next stage of the analysis will be aimed at predicting the evolvement of low back pain from acute to chronic already at early stages using supervised machine learning techniques.

Physical examination

The physical examination will first include neurological screening to ascertain that the subject is not presenting with radicular signs or signs of upper motor neuron lesions. Clinical evaluation will be performed as follows:

1. Lumbar range of motion: flexion, extension, side flexion, and rotation movements with bubble goniometer. Any aberrant movements will be recorded.
2. Functional test – sit-to-stand test (STS): Patients will be asked to perform five transitions from sit to stand as quickly as possible; the time to perform these five repetitions is the test result. The sit-to-stand transition is considered to be a mechanically demanding physical activity in daily life.55 For the first 2 weeks, participants will be asked to perform five sit-to-stand transitions each morning while they wear the ActiGraph and rate the severity of their pain. Accelerations of sit-to-stand transitions will be recorded for further analysis.

Questionnaires:

All questionnaires will be delivered by App (to complete)

1. Demographics – A personal questionnaire including age, sex, educational level, marital status, height, weight, duration of pain, working status, days missed at work due to LBP, satisfaction from work, past medical history, and drug consumption will be completed.
2. Pain severity will be recorded by the participants, who will be asked to rate their level of pain on an 11-point Numeric Pain Rating Scale (NPRS), with higher scores indicating greater pain. Participants will be asked to rate the current, worst (highest), and best (lowest) pain intensity ratings over the past 24 hours. The mean of the ratings will be analyzed.56 In addition, the participant will be asked to rate the pain when he/she gets out of bed. The frequency of low back pain during the previous 24 hours will be recorded. Study participants will be asked to describe the frequency of their low back pain, using the descriptors “always”, “usually”, “sometimes”, “rarely”, or “not at all”. 57
3. The Modified Oswestry Disability Index (MODI) will be used to assess disability associated with LBP. The MODI includes 10 questions regarding activities likely to be affected by LBP, such as walking, standing, sitting, and lifting. The actual score is presented in a percent format (0-100%): the higher the score, the greater the disability associated with LBP.58
4. A Fear-Avoidance Beliefs Questionnaire (FABQ) questionnaire will be completed to assess the participant’s beliefs as to the potential harm of several physical and work-related activities. The questionnaire comprises two subscales: a physical activity subscale (FABQ-PA) of four items and a work subscale (FABQ-W) of seven items. A higher score on either subscale indicates a greater level of fear.59,60
5. The International Physical Activity Questionnaire Short Form (IPAQ-SF) will used to evaluate physical activity level before the acute LBP event. The questionnaire includes four generic items about different exercise intensities (vigorous, medium, walking, and inactivity). The IPAQ is used as a comparable and standardized self-report measure of habitual physical activity.61

Data analysis

* Descriptive statistics performed on each study research cohort at Baseline will include all measured variables (demographic, anthropometric, level of activity prior to the acute phase, socio- and psychological state, and acute LBP severity).
* Primary investigation of the difference between those subjects who become CLBP and those who do not will employ a two-independent sample t-test or a χ2 test performed for each time point (variables include functional test immune profile, disability and pain severity level, and activity level using actigraphy).
* Multiple logistic regression models will be used to predict those subjects who will develop CLBP from measurements taken at Baseline and/or other time points. A stepwise procedure will be performed in order to achieve the best combination of variables to predict the CLBP patients.
* Sequences of measurements will be used to track changes in movement and pattern behavior by applying sliding windows of various sizes in order to identify meaningful temporal behavior patterns and their evolution over time.
* The immune system profile will be defined by clustering of the cell expression that will be explored through the time points to track changes. For the clustered variable, a mixed model will be fitted in which the cluster variable representing the immune system responses is the dependent variable, time is a fixed factor, and the patient is the random factor. The immune system profile will be measured at 3-5 time points and will be included in the model.
* In order to compare the trajectory of the movement patterns, a mixed model will be applied in which change in movement and pattern behavior is the dependent variable, and time and group viable (CLBP or not) are fixed factors.
* In order to compare the trajectory of immune profile, a mixed model will be applied in which changes in the immune system cluster are the dependent variables, and time and group viable (CLBP or not) are fixed factors.
* A mediation model will be applied in order to test whether the immune system mediates between exercise (number of steps and intensity) and CLBP.

**C.3. Preliminary Results**

The preliminary section demonstrates that the researchers in this proposal have the experience to perform the study and collect the data as proposed in the Methodology section.

**C3.1 Effectiveness of physical therapy for CLBP**

Recently, one of the PIs (GD) conducted apilot study to examine the effectiveness of physical therapy on CLBP. This study included 21 individuals (mean age 48.7±12.3) with CLBP admitted for physical therapy treatments at Maccabi Healthcare Services. All participants completed clinical physical examinations performed by experienced physical therapists. In addition, self-reported questionnaires for pain and function assessment were assessed at this session and at the end of intervention. Intervention included physical therapy treatments twice a week for 4 weeks (a total of 8 treatments). A follow-up telephone interview was conducted 3 months later for long-term assessment to grade the pain level of the participant.

The results showed a significant decrease in pain level, and an increase in function and fear avoidance following the physical therapy treatments (Table 1(. The long-term decreased level of pain was also evident, remaining at the 3-month follow-up 2.71 (±1.85).

The results of this pilot clinical study indicate that the research is feasible, the questionnaires and physical examinations are all known to the investigator (GD), and she has the ability to recruit patients, perform adequate examination for LBP, and follow them for a few months.

**Table 1**: **Outcome Measures of Pre- and Post-treatment**

|  |  |  |  |
| --- | --- | --- | --- |
| **Questionnaire/Scale** | **Pre-treatmentM(±SD)** | **Post-treatmentM(±SD)** | **P-value** |
| Oswestry disability index questionnaire | 22.57(13.9) | 13.52(11.0) | <0.001\* |
| Numerical pain rating scale | 3.76(2.1) | 2.38(2.03) | 0.009\* |
| Fear-avoidance beliefs questionnaire | 36.76(21.2) | 23.12(19.7) | 0.003\* |

\* significant difference between pre- and post-treatment.

**C3.2** **Ability to characterize immune cells at high resolution in blood using cytometry by time-of-flight (CyTOF)**

CyTOF is a powerful technology that allows simultaneous quantification of a large number of different cell types. In the case of the immune system, high resolution quantification of the many different cell types of the immune system, each with its own function, is possible (Fig. 2). CyTOF measures the abundance of metal isotope labels on antibodies on single cells using mass spectroscopy. It allows many more molecules to be used as probes than with traditional fluorescent label-based cytometry. CyTOF assays now routinely use 45 different labels to both cell surface and intracellular components simultaneously, allowing for broad immune system quantification of cell type and function.

**Figure 2: CyTOF captures a high-resolution system-wide snapshot of immune cell frequency and function.** Human PBMC measured by CyTOF using a combined cocktail of 37 antibodies staining against cell type, cytokine and cytokine receptor markers; analyzed and visualized using the SPADE algorithm. Cell-type marker antigen abundances define each individual cell measured as a single point in a 37-dimensional space. Cells are clustered into one of 200 clusters (nodes) connected by cluster similarity to form a tree-like structure. In this case, the intra-cellular expression of INFγ is shown (9 other cytokines were simultaneously measured in each cell). Low-resolution cell subsets were manually annotated and are shown by "bubbles" encircling matching fine-resolution cell subset nodes. Following stimulation, IFNγ is expressed broadly yet selectively across many cell types of the immune system, most prominently in CD8+ and CD4+ effector T-cells. Such data provide fine-grained measurements for individual response prediction.

**C.3.3** **Accelerometer-derived movement patterns**

We are experienced with examining movement characteristics in different populations (soldiers, cyclists, and people with diabetes) using warble devices for long periods of time: 32 weeks, 3 days, and 7 days, respectively). By using unsupervised machine learning techniques (clustering), we were able to separate populations of soldiers into a group of soldiers who were injured during basic training and a group that completed basic training without injury. Following this, we trained a classifier for predicting the risk of injury from early-stage data, with encouraging results.62

As part of a study conducted by Yalom-Peri as a PhD student under the supervision of CukiermanYaffe, T and Einat Kodesh), and in collaboration with Prof. Tsvi Kuflik, a pilot study was carried out with people with diabetes wearing actigraphy devices (ActiGraph wGT3X-BT) simultaneously with continuous glucose monitoring. We found that movement patterns of people with low physical capacity differ from others with a higher variability expressed by the root mean square of the X, Y, and Z axes. In addition, this group spent less time performing moderate to vigorous physical activity and had fewer daily steps compared to other groups.63 With this study, we gained expertise in data-derived continuous accelerometer collection (24 hours for 7 days) in free-living condition measurement and anelasticities.

A third, on-going study focuses on the behavior patterns of cyclists during training and competition, correlating the level of effort with road conditions (speed, elevation change) to assess the cyclists' capabilities and, in the future, to suggest guidelines for improved training.

We will further collaborate with Prof. Tsvi Kuflik (a full professor in the Department of Information Systems in which, among other things, he is researching the use of wearable devices to identify training patterns and predict injuries) on automatic analysis of the sensor data that will be collected, to identify groups of similar participants (given their behavior patterns) and to predict the likelihood of developing CLBP.

**C.4. Available resources**

Dr. Dar and Dr. Kodesh are full academic staff members (senior lecturers) in the Department of Physical Therapy, Haifa University.

**Dr. Dar** is an experienced physical therapist and anatomist, and carries out research in basic and functional science. She has considerable experience and numerous publications in spinal and back pain research, and all assessment and physical therapy treatments are well known to her. In her PhD thesis, she studied the sacroiliac joint with respect to its anatomy, function, and pathology, uncovering the unique phenomenon of sacroiliac joint bridging. She also studied the human spine with respect to the evolutionary process and to different spine pathologies. Dr. Dar has performed several clinical studies on patients admitted to physical therapy clinics with different orthopedic disorders (knee osteoarthritis, neck pain, chronic ankle instability), and also studies sport injuries and functional tests.

**Dr. Kodesh** is an exercise physiologist and physical therapist. Her areas of expertise include exercise physiology, physical therapy, and the interaction between the two. Her professional and academic training includes research in the field of performance, injuries, and the physiological response and adaptation to acute exercise and prolonged training under conditions of both health and disease. In her thesis and postdoctoral training, she studied and published articles on immune responses to exercise in humans and animal models Dr. Kodesh’s training and experience in basic and applied sciences are particularly relevant to the proposed study.

The immune system profiling panel will be constructed by the Cytometry Center in the Biomedical Core Facility of the Rappaport Faculty of Medicine at the Technion, headed by Dr. Amir Grau (letter of support is attached). The samples will be stained with the antibody panel and then run on the Helios™ mass cytometer (Fluidigm Inc.). Data analysis will be performed using Cytobank software (Beckman Coulter) and R software by students of Dr. Dar and Dr. Kodesh with guidance by the service center and oversight of the Shen-Orr lab at the Technion (see letter of support).

Movement patterns will be analyzed in collaboration with Prof. Tsvi Kuflik (letter of support is attached). Prof. Kuflik is a full professor of information systems and a former head of the Department of Information Systems. His research focuses on user modeling and intelligent-user interfaces. He is researching the potential of using advanced stationary, mobile, and wearable technologies to provide personalized services to their users. Prof. Kuflik integrates wearable technology, which provides continuous and synchronous recordings of movement, with physical activity behavior. With machine learning analysis, Prof. Kuflik works to characterize physical activity and gain insight into health behavior, health status, and musculoskeletal injuries.

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