**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, reduced quality of life and leads to higher medical and non-medical related costs for patients, employers, and health care providers. CLBP is usually defined as pain that lasts more than three months. **Establishing a predictive model for the transition from acute to CLBP could have significant personal, social and economic benefits enabling clinicians accurately predict the patients who are at a high risk of developing CLBP in the early stages of the condition.** Prognostic models that consider patient characteristics may provide evidence-based input for treatment decisions.

During the proposed study, we will use sophisticated technology to examine new major 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 mediates pain level and might affect LBP chronicity process.

We will examine these aspects in few time points during three month process and compare between those who eventually developed CLBP and those who not. Thus, enable us to determine the earliest time point of the transition toward chronicity.

**The overall hypothesis is that immunological and movement pattern biomarkers may contribute to the understating of the transition from acute to chronic low back pain and allow to predict those who developed CLBP.**

To establish this, we will examine participants with 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 (function Sit-to-stand test, spine range of motion) and blood test. Each participant will receive an accelerometer (Wireless ActiGraph GT3X) to wear continuously for a period of 3 months in order to monitor the movement pattern and physical activity behavior. Participants will rate their perceived pain, fatigue, and movement limitations in a mobile application.

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

Blood tests and physical examination will be taken at different time points:0 2, 4, 8, and 12 weeks. Participants will be defined as CLBP or control if they have pain after 3 months or not and comparison between groups will be performed for all measurements.

**Expected significance:** Predictionmodel for the transition from acute to chronic LBP is essential and important to the understanding of LBP entity. This will further improve treatment and decrease health system costs.

**Research program**

1. **Scientific Background**

Chronic low back pain (CLBP) is a complex disease with high heterogeneity in symptoms, level of pain, function, and more, which further challenges decision-making during the adaptation of appropriate treatment. In addition, the extent to which a patient is at risk of developing chronic LBP is essential to make treatment decisions.

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

***Low back pain prevalence***

Low back pain (LBP) is the most common disorder that causes disability, functional decline, and reduced quality of life. LBP leads to higher medical and non-medical related costs for patients, employers, and health care providers. In the United States, treatment for LBP and related spine disorders represents the most expensive medical problem and 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, where the reminded 10% of the patients are specific diagnosed with malignancy, radiculopathy, stress fractures, spinal stenosis, spondylolisthesis, or blood vessels 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 chronic low back pain (CLBP) is between 4-40% 1,9 Chronic pain is defined as pain that is not relieved in 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 for many studies, trying to identify risk factors for chronicity process with the aim to hamper 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 patient’s improvements of symptoms, and have relationships 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, depression.14,15

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

***Immune system and pain***

Previous evidence showed 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 level in the plasma were associated with the 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 an imbalance between pro-inflammatory and anti-inflammatory mediators contributes to the pathophysiology of CLBP. The level of pro cytokines is 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 recent study that characterized and compared inflammatory profile of acute and chronic LBP patients, it was found that the production of pro inflammatory cytokines TNF alpha, IL -6, IL-1 beta, was elevated and the anti-inflammatory cytokines IL-10 was reduced in both LBP patients.25 The differences between acute and chronic phases of LBP included the following – higher production of TNF alpha, IL-1 RA, and sTNFR2 in the CLBP while during the acute phase higher IL-2 interferon Gama was observed.25

More studies that support these findings need to be further investigate in order to understand the transition from acute to chronic LBP.

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

In recent years, technological development in profiling the immune system allows broad high-resolution profiling of immunity which was not possible prior. This easily encompasses simultaneous measurements of hundreds of different immune component (cell types, cytokines, mRNA, and others).28 This new technology allows a deeper understanding of how the different features related one to another, overcomes the high variance observed when measuring only few representing component (e.g. C reactive protein and IL6)29. This can provides comprehensive targets for the prediction of clinically relevant information. More recently a novel fluorophores and laser systems, driving the discovery of new immune cell subclasses as well as important functional states.30 This may navigating an individual’s decision in predicting disease or conditions early or guiding treatment. For instance, immune-based blood biomarkers have been identified for hip surgery recovery time and pain responses.31

We believe that a more extensive immune profile that allows assessing the complexity of the immune system, in a more comprehensive way, would yield better information about the role of 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 level of physical activity intensity and movement pattern during the day. As far as we know, only few studies37,38 continuously measured physical activity by tri-axial accelerometer for 1 week during the admission phase for CLBP. Collecting movement data by accelerometer allows for objectively, monitoring and evaluating 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 exercises were demonstrated as 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 and the effect these movement pattern on transition still need further investigation.

***Physical activity and immune system***

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

The immune system activation is a response to a stressor such as exercise aiming to restore cellular homeostasis. The immune system is very responsive to exercise,47 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 reestablishment of cellular and tissue function.46

Exercise training reported to counters inflammation elements of some disease process49 characterized in part by high inflammation, oxidative stress, and immune dysfunction, stimulating many cellular and molecular changes throughout body tissues that promote anti-inflammatory and antioxidant responses, and augment immunosurveillance.47

The individual mobility behavior can influence the immune system profile50 which mediates pain level and thus affecting the LBP chronicity process.

1. **Research objectives & expected significance**

Chronic low back pain 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 available optimal treatment. As such, it is essential to identify additional risk factors and use 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.

During 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 in few time points during the three month process and compare between those who eventually developed CLBP and those who not. Thus, enable us to determine the earliest time point of the transition toward chronicity and develop 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

**As such, our specific aims are to:**

1. Compare demographic, movement pattern, functional, pain perception, psychosocial and immune clusters parameters between patients who developed CLBP to those who did not at different time points.

2. Define and cluster the changes in movement patterns, pain perception, function, and immune profile along three months follow-up from the acute event.

3. To identify the transition from acute to CLBP from immune profile and movement pattern.

4. To develop prediction model to identify patients at their acute phase who are at risk to develop 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 chronic LBP.

Examining these factors, combined with known, affecting aspects of LBP, will establish further observation of the CLBP. Mapping this relationship will improve clinical decision making and open the door to achieving a personalized and effective therapeutic plan which further improve 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:**

The overall hypothesis is that immunological and movement pattern biomarkers may contribute to the understating the transition from acute to chronic low back pain and allow to predict those who developed CLBP.

**Specific hypotheses:**

* + - 1. Differences in demographic, movement pattern, functional, pain perception, psychosocial and immune clusters parameters will be found between patients who developed CLBP and those who did not at all time points.
      2. After one months, those who will develop CLBP will demonstrate: higher pain perception, lower functional score, lower physical activity, higher medial-lateral (y axis amplitude) movement, higher movement during sleep and higher pro-inflammatory status in immune system.
      3. Transition from acute to CLBP, will be demonstrated via the immune system and movement pattern, already after 2 weeks from acute event
      4. Immune system and movement pattern variables will predict the transition for CLBP.

**Research design & methods:**

In this study we chose to learn about LBP as it is one of the major musculoskeletal disorders which causes high health system costs and is still a challenge for physicians to treat with no specific solution. Developing and examining a new model for LBP chronicity will gain further insight into the understanding of CLBP pathophysiology and may lead to the identification of novel targets for treating symptomatic CLBP patients and adjustment of personalized treatment. We believe that our new aspects of biology-immune system responses and movement pattern changes during the chronicity path will allow better practical intervention and wiser resource distribution to reduce CLBP incidence.

**Research plan**

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

The participant will be followed-up longitudinally for a period of three months. After completion the study period, participants will be divided into two groups: (1) those who developed chronic low back pain (i.e., CLBP group); (2) those who did not (i.e., control group).

The research will be submitted for approval of the Helsinki committee in Maccabi health care services.

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

***Participants***

This prospective study design 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 the required sample size to achieve 80% power for detecting a 0.4 effect size, at a significance criterion of α = .05, was *88.*  Thus, we obtained sample size of *100* (+20 for the exploratory phase as will describe later) to test our study hypothesis.52

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

Subjects will be excluded from participation if: specific LBP (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 are currently being treated.

***Research procedure:***

Study procedure is described in Figure 1. Participants will be recruited to the study and perform a baseline examination (T0) as earlier to their low back pain episode onset and no more than 2 week after. A 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 abilities 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 during the day on the waist, and during the night (when sleeping) on the wrist. In general, the accelerometer will be worn at all times for a period of 3 months in order to monitor the movement pattern and physical activity behavior. By using a mobile application, participants will be asked to rate their perceived pain, fatigue, and movement limitations using a visual analog scale (grades 1-10) every day for the first 2 weeks. During this 2-week period, the researcher will perform weekly phone calls with participants to confirm their compliance with the study requirements and tasks.

Blood tests and physical examination will be taken at different time points:0 2, 4, 8, and 12 weeks Participants will be defined as CLBP or Control if he/she still has pain after 3 months or not.

**Figure 1: Outline of research design**

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***Measurements***

Blood test for immune profile:

Since the processing of the innovative technology of immune profile is very expensive, the immune system profile analysis will be composed of two phases: Exploratory phase (Fig. 1) will enable 5-time points (T0-acute phase on admission for physician help; T1-2 week after T0, T2-4 week after T0; T3-8 weeks after T0 and T4- 12 weeks after T0) of blood samples 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 with no CLBP) will be monitored and a full analysis of all measurements (5-time points and all related variables) will be performed. This will allow us to detect the significant time points which most likely will be important for research aims that may suggest the trajectory of chronicity. The 2nd phase aimed to prediction analysis and will examine the three most significant selected time points and variables as detected in the exploratory phase in a larger sample size (100 participants). All blood samples will be stored at -20 degree (as will be described under the description of blood test) and will be analyzed only after 10 participants from each group (CLBP /control) will be defined after 3 months. According to data analysis of the first 10 participants from each group the time points that will indicate the 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 named mass cytometry (commercial product is called a “CyTOF”) that enables high-resolution monitoring of an individual’s immune system.28,53 The CyTOF is a single cell proteomics antibody-based technology allowing to profile ~40 proteins per cell (see Preliminary Results). The advantage of mass cytometry is that many molecules may 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 into sodium heparin tubes and transferred into Prot1 ™ proteomic stabilizer (SmartTube™ Inc) at 1:1.4 ratio incubated at room temperature for 10 minute. Tubes will then be stored in -80 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 Two ml blood will be drawn directly into a PaxGene RNA stabilizer tube for gene expression. Paxgene tubes will be stored at -80 until processing. RNA will be extracted using PAXgene Blood RNA Kit (Qiagen) and gene expression will be analyzed.

Daily physical activity and movement patterns

Daily physical activity (PA) will be collected 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 14g. Participants will be asked to wear the ActiGraph wGT3X-BT sensor during waking hours on the waist and on the wrist during sleep time. The following data will be collected: Raw Acceleration (the physical movements of each patient in three-dimensional space in 0.1 s intervals.), activity intensity, steps, total movement, total sleep time (TST), sleep efficiency, wake after sleep onset (WASO), sleep fragmentation. The data that will be analyzed to produce ongoing behavioral patterns (behavior over time) as well as cumulative data that will include the amount of time sitting, standing, walking, and lying down, walking duration, number of walking episodes.

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

The data that will be collected will be analyzed (abstracted and integrated) and used to determine the individual type and level of physical activity as well as sleep patterns, as well as for identifying groups of patients with similar characteristics and hopefully be able to distinguish between patients with acute to chronic low back pain (using unsupervised machine learning techniques – clustering). The next stage of the analysis will aim 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. Than 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 are asked to perform five transitions from sit -to -stand as quickly as possible with the time to perform these five repetitions is the test result. The Sit-to-Stand transition is considered to be mechanically demanding physical activity in daily life.55 In the first 2 weeks, in the morning of each day, participants will be asked to perform five sit-to-stand while they wear the ActiGraph and rate their pain severity. Accelerations of sit-to-stand transitions, will be recorded for further analysis

Questionnaires:

All questionnaire will be delivered by App (to complete)

1. Demographics - For participant's characterization demographic, the personal questionnaire will be fill including age, gender, educational level, marital status, height, weight, duration of pain, working status, days missed at work due to LBP, satisfaction from work, past medical history, drug consumption.
2. Pain severity - for assessing pain severity Numeric Pain Rating Scale, NPRS: will be

recorded by the participants, which will be asked to rate their level of pain on an 11-point numeric pain rating scale. The higher the grade, the greater the pain. Patient 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 analysis.56 In addition, he/she will be asked to rate the pain when he/she get out of bed. The frequency of low back pain during previous 24 h will be rated. Study participants were asked to describe the frequency of their low back pain, using the descriptors “always, “usually,” “sometimes,” “rarely,” or “not at all.”57

1. Disability level associated with LBP: to assess disability the Modified Oswestry Disability Index (MODI) will be used: 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 percentage format (0-100%); the higher the score, the greater the disability associated with LBP.58
2. To assesses the subject’s beliefs as to the potential harm of several physical and work-related activities. A Fear-Avoidance Beliefs Questionnaire (FABQ) questionnaire will be filled. The questionnaire consists of 2 subscales: a physical activity subscale (FABQ-PA) of 4 items and a work subscale (FABQ-W) of 7 items. A greater score on either subscale indicates a greater amount of fear.59,60
3. To evaluate the Physical activity level before the acute LBP event the international physical activity questionnaire short form (IPAQ-SF) will used. The questionnaire includes 4 generic items about different exercise intensities (vigorous, medium, walking and inactivity) The International Physical Activity Questionnaire (IPAQ) is used as a comparable and standardized self-report measure of habitual physical activity.61

Data analysis

* Descriptive statistics will be performed for characterized the study research cohort at baseline including all measures variables (demographic, anthropometric, questioner level of activity prior to the acute phase, socio and psychological state, and acute LBP severity)
* To primary investigate the difference between those that become CLBP to those that do not, a two-independent sample t-test or χ2 will perform for each time point (variables include: questioner functional test immune profile, disability and pain severity level, and activity level using actigraphy)
* To predict those who will develop CLBP multiple logistic regression models will be used with measurements taken at the 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.
* For tracking changes in movement and pattern behavior, sequences of measurements will be considered by applying sliding windows of various sizes in order to be able to identify meaning temporal behavior patterns and their evolution over time
* For tracking the changes in the Immune system: the immune system profile will be defined by clustering of the cell expression that will be explored through the time points. For the clustered variable a mixed model will be fitted where the cluster variable representing the immune system reaponses 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 performed where the change in movement and pattern behavior is the dependent variable; Time and group viable (CLBP or not) is a fixed factor.
* In order to compare the trajectory of immune profile. A mixed model will be performed where the changes immune system cluster is the dependent variables; Time and group viable (CLBP or not) is a fixed factor.
* In order to test if the immune system mediates between exercise (number of steps and intensity) and CLBP, a mediation model will be performed.

**C.3. preliminary results**

The preliminary section demonstrated that the researcher pf this proposal have the experienced 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 to physical therapy treatments at Maccabi Health 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 end of intervention. Intervention included physical therapy treatments twice a week for 4 weeks (total of 8 treatments). A follow telephone interview was performed 3 months for long term assessment to grade the pain level of the participant.

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

The results of this clinical pilot study indicate that the research is feasible, the questionnaires and physical examinations are all known to the investigator (G.D) with the ability to recruit patients, performa adequate examination for LBP and follow up after them for few months.

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| **Table 1**: Outcome measures of pre and post-treatment | | | |
|  | **Pre-treatment**  **M(±SD)** | **Post-treatment**  **M(±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 technology that allows powerful quantification of a large number of different cell-types simultaneously. In the case of the immune system, this enables high resolution quantification of the many different cell-types of the immune system, each with its own function (Fig 3). 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 fluorescence label based cytometry. CyTOF assays now routinely use 45 different labels simultaneously both cell surface and intracellularly, allowing for broad immune system quantification of cell-type and function.

**Figure 3: CyTOF captures a high-res 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 showed 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 provides fine grained measurements for individual response prediction.**

C.3.3 **Accelerometer-Derived Movement Patterns**

We are experienced with examining movement characteristics in a different population (soldiers, cyclist and people with diabetics) using warble devices for a long period 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 to a group os soldiers that got injured during basic training and a groups that completed basic training without injury. Following this step we trained a classifier for predicting the risk of injury from early stages data with encouraging results.62

As part of PhD student study conducted by Yalom-Peri under the supervision of (Cukierman–Yaffe, T and the PIs EK), and in collaboration with Prof. Tsvi Kuflik, a pilot study was done 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 are different from other with a higher variability expressed by the root mean square of the X, Y, 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 By doing this study we gain expertise in data derived accelerometer collection continuously (24 hours for 7 days) in free-living conditions measurement and anelasticities.

A third, on-going study focusses on the behavior pattern of cyclists during training and during a competition – correlating the level of effort with road conditions (speed, elevation change) for assessing the cyclists' capabilities and in the future, suggesting guidelines for improved training.

We will further collaborate with Prof. Tsvi Kuflik (a full professor of information systems department that works, among other things on, he is researching the use of wearable devices for identifying training patterns and predicting injuries) on automatic analysis of the sensors data that will be collected – for identifying groups of similar participants (given their behavior patterns) and for predicting the potential of developing chronic low back pain.

**C.4. Resources available**

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

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

**Dr. Kodesh** is an exercise physiologist and physical therapist. Her expertise areas of interest 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 postdoc training she studied and published immune responses to exercise in humans and animal models Dr. Kodesh 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 at the Biomedical Core Facility at 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).

The movement pattern 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 information systems department. His research focuses on user modeling and intelligent user interfaces. He is researching the potential of using stationary, mobile, and wearable advanced technologies to provide personalized services to their users. Professor 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 the physical activity and gain insight into health behavior, health status, and musculoskeletal injuries.

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