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Predicting bone regeneration with a simple blood test

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Abstract:

Cheng and colleagues reported previously unexplored correlations between circulating levels of immune cells and biomarkers, and bone regeneration that served as support for the construction of a model ensemble that can predict bone regeneration. If validated in humans, this tool could be valuable in the management of non-union fractures.

Keywords: bone regeneration, non-union fracture, myeloid-derived suppressor cell, B-cell

Worldwide, around 9 million fragility fractures, caused by osteoporosis, occur each year. This figure is even greater if everyday life and road accidents or battlefield injuries are accounted for. Due to the self-regeneration of bone tissue, in most cases, the fracture-related pain subsides, and the inconvenience vanishes as soon as the plaster cast is removed. However, for 5-10% of individuals with a broken bone, the fracture will fail to heal under the usual care and leads to a delayed union or even worse non-union [1]. Fractures which progress to non-union are difficult to treat, with many leading to marked disability from pain to functional impairment. In addition to individual patient disability, non-union is associated with increased healthcare utilization and cost.

Physiological fracture repair requires several steps that lead to the formation of a hematoma that bridges the bone fragments and provides support for the formation of a soft callus. Hematoma remodeling is associated with a local infiltration of immune cells coordinating the formation of the soft callus. Soft callus is made of new connective tissue, blood vessels and cartilage. Later, the soft callus transforms into a hard callus that consolidates the fracture site and leads to the first template of bone tissue that is secondarily remodeled to a stronger and better organized bone tissue [2].

Various factors augment the risk of non-union fracture: severe bone fracture, mechanical instability, chronic metabolic diseases such as diabetes and osteoporosis, ageing, smoking, alcohol abuse, and use of several drugs such as opioid, anticoagulant, or anti-inflammatory molecules. Current treatment of non-union bone fractures usually involves surgery to stabilize the area and the insertion of a bone substitute, either as a bone graft or a synthetic scaffold, to promote and stimulate angiogenesis and osteogenesis. Systems to deliver growth factors or stem cells have been developed in the last two decades to repair small and large bone defects, respectively. However, the success of such approaches in vivo is inconsistent and it appears that the response of the local bone microenvironment is a key factor in the outcome of regenerative constructs [3].

Recent studies have reported that morbidity following severe trauma is usually associated with a dysregulated immune response. With this in mind, the recent study by Cheng and colleagues aimed to characterize the immune profile associated with bone regeneration in a preclinical rodent model of non-union fracture [4]. More specifically, their hypothesis was to investigate whether the early systemic immune response following traumatic injury could be predictive of long-term bone regeneration. In this elegant work, Cheng and colleagues used a rat model where a critical-sized bone defect was created at the femur mid-diaphysis; treatment with a BMP-2-loaded alginate scaffold was initiated either immediately or after 8 weeks, the latter mimicking the situation of non-union. Blood samples were drawn at 0, 1, 2, 4, 8, 9, 10, 12, 16, and 20 weeks of the study to perform a longitudinal follow-up of systemic changes in immune cell and cytokine profiles. At the end of the study period, and as expected, the amount of bone and the mechanical competency of newly-formed bone was lower in the non-union group than in the early-treated group.

Pooled univariate and multivariate analyses of circulating markers, at all study time points, highlighted unprecedented inverse correlations between circulating levels of CD11b⁺ His48⁺ myeloid-derived suppressor cells (MDSCs), CD68⁺ monocytes, interferon gamma-induced protein 10 (IP-10), and bone healing. On the other hand, circulating levels of B220⁺ B-cells and of interleukin (IL)-6 and

IL-13 were all positively correlated with bone regeneration. Independent analyses of tissue harvested at the time of animal sacrifice demonstrated consistent correlations with the blood immune profiles, suggesting that local tissue response could be monitored by measuring levels of these mediators. If only blood data obtained a week after initiation of treatment were considered, circulating levels of MDSCs, monocytes and IP-10 were still negatively correlated with bone healing whilst circulating levels of B-cells, T helper cells and IL-13 were positively correlated with bone regeneration.

In silico analysis of best parameter candidates for predictive bone regeneration revealed the pivotal role of MDSCs and of the regulatory cytokine IL-10 in almost 90% of all generated models, suggesting an unexpected relationship between local and systemic immune dysregulation in the lack of bone repair in non-union fracture. Previous investigations have shown that immune cells, and especially B-cells, massively infiltrate the fracture callus soon after injury, and locally contribute to the deposition of bone matrix and bone regeneration [5, 6]. In addition, growing evidence indicates that MDSCs can directly suppress B-cell differentiation and cytokine secretion [7, 8]. Therefore, the findings of the present study provide evidence that the dialog between B-cells and MDSCs may be indicative of an overall pro-regenerative versus anti-regenerative response (Figure I).

Several other groups have begun to explore strategies for local delivery of immunomodulatory agents, in order to speed up bone regeneration and repair, by targeting either macrophages or T cells [9, 10]. However, little is known about the role of MDSCs and B-cells in the context of bone regeneration. The work reported here underlies the importance of investigating the contribution of these cells to this process.

Interestingly, model ensemble prediction, built on data available a week after initiation of treatment, demonstrated a high predictive power ($R^2=0.9255$) for bone regeneration. Although the predictive power of this ensemble remains to be validated in humans, this finding may pave the way for the development of an algorithm that could predict bone healing outcomes with a simple blood test. Development of such an approach would be a valuable tool and would represent a major step forward in the care of patients with a non-union fracture.

In conclusion, this enlightening article provides new mechanistic insights into the role of systemic immune dysregulation on bone healing in a rat model of chronic non-union fracture. The largely unexplored role of MDSCs and immune dysregulation offers new approaches to care for patients with non-union fracture, potentially serving as a predictive tool for bone healing outcome and as a novel therapeutic target to enhance bone repair.

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Figure captions

Figure I: **MDSC and B-cell interplay is essential for bone regeneration.** Rapidly after injury, B-cells invade the soft callus and participate in the differentiation of the soft callus into a hard mineralized callus. Reduced function of B-cells by MDSCs and secreted mediators, such as IL-10, contribute to reduction of bone self-repair and non-union fracture.

