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## Bone Marrow Tinctures for Cardiovascular Disease: Lost in Translation

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Warburg's Tincture was a blockbuster anti-fever remedy in the 19th century. It was invented by Dr. Carl Warburg to treat a broad range of fevers, achieving widespread usage in the British Empire, the Austrian Empire and also in the United States. Many renowned physicians prescribed it, but others were envious of Warburg's success and not too happy about the fact that he kept its formulation secret. In 1870, Warburg finally revealed the secret ingredients and gave permission to Professor W. C. Maclean, one of the most ardent supporters of the remedy, to publish them in the *Lancet*<sup>1</sup>: The elusive tincture contained many components, such as Rhubarb root, Angelica seeds, gentian root, Saffron, Fennel seeds, Myrrh, Camphor among others and, unsurprisingly, quinine. In his letter to the *Lancet*, Maclean effusively praises the tincture, but he also emphasizes that its healing power was not just due to the known antipyretic quinine, but that it was the unique composition of various extracts that was responsible for its efficacy. In the 21st century, few physicians would prescribe such tinctures, which are plant or animal extracts. The idea of using such extracts which contain a large array of substances, some active and some inactive, goes against the tenets of modern medical practice, in which we strive to define and titrate every single therapeutic agent to optimize efficacy. There is, however, one notable exception to this practice in an emerging area of cardiovascular medicine: the use of bone marrow mononuclear cells.

An animal study in 1999 suggested that mononuclear cells extracted from the bone marrow and injected into the heart could be used to augment cardiac function after a myocardial infarction<sup>2</sup>. These results were translated into the clinical setting at an astonishingly rapid pace, as evidenced by the publication of a small clinical study in 2002 showing that intracoronary infusion of bone marrow mononuclear cells (BM-MNCs) after an acute MI could be performed safely without major complications and might even reduce infarct size and improve left ventricular function when compared to standard therapy for MI<sup>3</sup>. These early findings caused a considerable amount of excitement in the field of cardiovascular regeneration because they demonstrated that stem cell therapies to augment cardiac function

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were not only feasible but even appeared to be efficacious leading to multiple subsequent studies in patients who had either suffered an acute MI or had chronic ischemic heart disease. The enthusiasm was further buoyed by an animal study which suggested that hematopoietic stem cells in the bone marrow could differentiate into functional cardiomyocytes<sup>4</sup> and this was proposed as one conceivable mechanism by which the BM-MNCs were improving cardiac function. However, there was one key problem with the use of BM-MNCs, which may not have received the degree of attention that it ought to have. BM-MNCs are a rather heterogeneous combination of cell types, mostly consisting of lymphocytes, but they can also contain monocytes, endothelial cells, mesenchymal stem cells, pericytes and mesenchymal lineage progenitors derived from the mesenchymal stem cells such as pre-adipocytes or osteoblasts. In fact, hematopoietic stem cells represent only a tiny fraction (roughly 1% or less) of the total BM-MNCs. Most clinical studies did not characterize the relative proportions and respective activity of the various cell fractions contained in each of the heterogeneous bone marrow extracts. They instead focused on standardizing the total number of infused or injected cells, hoping that one or more of the cell types in the BM-MNC extract would exert therapeutic benefits and that the individual differences in BM-MNC composition and activity would not confound the therapeutic efficacy. Thus, even though the BM-MNCs had not been extracted with ethanol (the classical way to make a tincture), the heterogeneity and lack of clear characterization of the various components of the bone marrow extracts was reminiscent of 19th century tinctures.

In hindsight, one may wonder why the use of BM-MNCs became so popular despite the prevailing knowledge that the majority of injected cells were lymphocytes and not stem or progenitor cells. The convenience of obtaining an autologous bone marrow extract from a patient and infusing it without clearly characterizing and separating its cellular components might have played a role. The fact that the expression “bone marrow” automatically conjures up images of stem cells, in light of the tremendous success of hematopoietic stem cell transplantation of with stem cells, may have also contributed to the popularity of using BM-MNCs in these early studies. If they had been correctly referred to as “Infusions of lymphocytes combined with multiple other cell types”, more questions might have been raised about whether they were indeed the most appropriate treatment to study regenerative therapies in cardiovascular disease.

Even though additional small clinical studies supported the use of BM-MNCs via intracoronary or intramyocardial injections, a 2006 study published in the *NEJM* showed no evidence of improved cardiac function following BM-MNC infusion after an acute MI<sup>5</sup>. Furthermore, one of the purported mechanisms of BM-MNC action, the differentiation of hematopoietic stem cells into cardiomyocytes<sup>4</sup>, also became an issue of controversy when a subsequent study found no evidence of cardiomyocyte differentiation<sup>6</sup>. This suggested that the observed benefits seen in the BM-MNC studies may not have been the direct result of hematopoietic stem cells differentiating into cardiac cells, but possibly due to other unidentified cells contained in the BM-MNC extracts. In addition to these questions about the unclear mechanism of BM-MNC action, it became apparent that BM-MNCs did not exhibit long-term engraftment in the heart<sup>7-8</sup>.

None of the clinical studies using BM-MNCs in acute MI or ischemic heart disease were powered to detect endpoints such as mortality, most enrolled only 10–100 patients and used indicators of LV function as endpoints. A comprehensive meta-analysis of 50 BM-MNC randomized controlled trials and cohort studies (with a combined enrolment of 2625 patients) was conducted in 2012 and found that BM-MNC therapy resulted in a statistically significant, but rather modest left ventricular ejection fraction (LVEF) improvement of 3.96%, and similar benefits in other indicators of left ventricular function<sup>9</sup>.

Soon after the publication of this meta-analysis, the results of the randomized controlled TIME trial were released, which enrolled 120 patients with an anterior STEMI and assigned them to either early (3 days after STEMI and reperfusion) or late intracoronary BM-MNC infusions (7 days after STEMI reperfusion), or the respective placebo infusion groups. The TIME trial found no significant benefit of either early or late BM-MNC infusions, as assessed by cardiac magnetic resonance imaging of LV function at 6 months post cell therapy<sup>10</sup>. The TIME trial once again highlighted the inconsistent efficacy of BM-MNCs, which had also been observed in the meta-analysis<sup>9</sup>, but its results were also criticized. One key criticism leveled at the TIME trial was that the TIME investigators had employed an automated method to extract the BM-MNCs instead of the more traditional Ficoll gradient centrifugation method traditional used by previous trials which had shown benefits of BM-MNCs<sup>11</sup>. A second criticism was that the isolation method of the TIME investigators had exposed the BM-MNCs to the anticoagulant heparin, which might have impaired the activity of the infused BM-MNCs<sup>11</sup>.

The results of the SWISS-AMI trial published in this issue of *Circulation* by Sürder and colleagues may be able to address these concerns<sup>12</sup>. The SWISS-AMI trial enrolled 200 patients with an ST-elevation myocardial infarction after they had undergone reperfusion therapy (primary PCI in most cases): 1) Control group, which consisted of optimal medical management, 2) Early BM-MNC therapy group, which received an intracoronary BM-MNC infusion within 5–7 days after the initial STEMI reperfusion in addition to optimal medical therapy and 3) Late BM-MNC therapy group, which an intracoronary BM-MNC infusion 3–4 weeks after the initial STEMI reperfusion in addition to optimal medical therapy. The investigators assessed LV function using cardiac magnetic resonance imaging at baseline and at 4 months for all enrolled patients. Their results show that there was no statistically significant difference in the LV ejection fraction between the three groups. The investigators used the density centrifugation method to isolate the BM-MNCs instead of the newer automated method used by the TIME investigators. The SWISS-AMI investigators used heparin during the bone marrow aspiration procedure to avoid clotting of the sample, but they avoided directly adding heparin to the isolated BM-MNC cell product, thus allaying concerns that the lack of efficacy was due to heparin treatment of BM-MNCs.

The SWISS-AMI trial has a number of key strengths, such as the randomized design, performing state-of-the-art cardiac magnetic resonance imaging to assess LV function on all patients at baseline and at a 4-month follow-up, using traditional BM-MNC isolation procedures, and the enrolment of 200 patients, which makes it one of the largest randomized BM-MNC trials in STEMI patients to date. The investigators also assessed the composition of the infused BM-MNCs, showing that roughly only 1% of the infused BM-MNCs were hematopoietic stem cells, whereas 46% of the cells were lymphocytes and 8% were monocytes, again underscoring that the vast majority of BM-MNCs are not stem cells.

It is important to note that the SWISS-AMI trial also has key limitations. The LVEF is only a surrogate measure for clinically meaningful endpoints, such as mortality, hospitalization for heart failure or need for revascularization and the trial was not powered to detect these more important endpoints. The vast majority of patients in all three groups were in NYHA class I and CCS class I at the follow up, thus indicating that the studied population had limited prevalence of heart failure and angina. Patients with more severe symptoms might have derived greater benefits from BM-MNC therapies. The study only followed the patients for four months and longer follow up might have revealed some statistically significant benefits. Lastly, the study only assessed the fractions of lymphocytes, monocytes and hematopoietic stem cells in the BM-MNCs, but not the proportion of mesenchymal stem cells, which have also been shown to exert a therapeutic benefit on LV function<sup>13</sup>.

Despite these limitations, the SWISS-AMI trial once again casts doubt on the idea that BM-MNC infusions are beneficial for MI patients. Even the comprehensive meta-analysis had shown only very modest benefits<sup>9</sup>, but in light of the newer negative TIME and SWISS-AMI data, cardiovascular researchers and physicians need to reconsider whether BM-MNC therapies should be pursued. One of the most likely explanations for the wide disparity and heterogeneity observed in the BM-MNC trials is the fact that each bone marrow extract contains varying proportions of cell types. Even though there is minimal evidence for adult BM-MNCs differentiating into functional cardiomyocytes, beneficial cell types in the bone marrow may release growth factors which prevent cardiomyocyte death or fibrosis, stimulate angiogenesis or activate resident regenerative cells contained within the heart. The large percentage of leukocytes in the BM-MNC might also result in the release of harmful, pro-inflammatory factors, as has been observed in monocyte-derived “early” endothelial progenitor cells (EPCs)<sup>14</sup>. Whether a BM-MNC infusion turns out to be beneficial, inert or harmful for any given patient probably depends on the relative proportions of the various BM-MNC cell types in that individual’s bone marrow.

There are calls for a large-scale BM-MNC trial<sup>11</sup>, such as the planned BAMI trial (NCT01569178), which intends to recruit 3000 patients and demonstrate that a single intracoronary infusion of autologous BM-MNCs reduces all-cause mortality in MI patients, hoping that it would definitively resolve the issue of whether or not BM-MNCs are efficacious. However, one needs to ask the question whether conducting such a large-scale BM-MNC trial in light of the recent negative results and newer scientific advances in the field of cardiovascular regeneration is even appropriate. When the first BM-MNC trials were conducted more than a decade ago, they were based on the scientific data that was available at that time. The use of a heterogeneous bone marrow gemisch in the hope that some cell type contained in the bone marrow extract would improve cardiac function might have been appropriate one decade ago. However, now we know about the lack of long-term engraftment of BM-MNCs, and we know that there are many other, better characterized and defined cell types that may be more effective at promoting cardiovascular regeneration.

Cardiac stem or progenitor cells can be obtained from a cardiac biopsy, appear to exhibit a true cardiomyogenic phenotype and have been safely used in early human trials<sup>15-16</sup>. Mesenchymal stem cells are a well-characterized subset of the BM-MNC fraction and clinical studies not only indicate safety but also some degree of efficacy<sup>13</sup>. Each of these cell types requires a more cumbersome isolation and culture procedure than the convenience of extracting BM-MNCs and re-infusing them into coronary arteries, but the use of these newer, defined cell types has a stronger scientific footing than the use of BM-MNCs. Even if a large-scale BM-MNC trial were to succeed and were to show a statistically significant modest benefit of BM-MNC infusions (as one might expect, based on the meta-analyses), we might still have difficulties explaining the underlying scientific mechanisms when using such poorly defined, heterogeneous BM-MNC extracts. The recent EDTA chelation therapy TACT trial is one example of a study with a statistically significant benefit, but with an unclear underlying scientific mechanism and it makes it very difficult to interpret its results<sup>17</sup>. For regenerative therapies to succeed, we need to ensure that large scale cell therapy trials are based on the latest and best scientific data and methodology available. We owe this to the study subjects, future patients and the scientific integrity of the field.

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