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Diabetes impairs mobilization of stem cells for the treatment of cardiovascular disease☆,★
A meta-regression analysis

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ABSTRACT

Background: Experimental diabetes impairs bone marrow (BM) mobilization of stem and progenitor cells involved in cardiovascular repair. We aimed to ascertain whether the presence of diabetes negatively affects the mobilization of stem cells induced by granulocyte-colony stimulation factor (G-CSF) in therapeutic trials for cardiovascular disease (CVD).

Methods: We conducted a meta-regression analysis of clinical trials published from 1997 to 2012 using G-CSF to yield BM stem cell mobilization in patients with CVD. We collected data on demographics, treatment regimen, degree of CD34+ cell mobilization, prevalence of diabetes and of other traditional risk factors. The primary aim was detection of a correlation between prevalence of DM and achieved CD34+ cell count after G-CSF treatment.

Results: We screened 227 articles, retrieved 96 for evaluation and retained 24 for the analysis of the primary end-point. There was a strong negative correlation between prevalence of diabetes and achieved CD34+ cell levels after G-CSF stimulation ($r = -0.68$; $p < 0.0001$), while there was no correlation with other traditional risk factors. A multiple regression analysis showed that the correlation between diabetes and mobilization was independent. In 13 articles reporting pre- and post-G-CSF cell counts, the increase in CD34+ cells was also negatively correlated with prevalence of diabetes ($r = -0.82$; $p < 0.0001$).

Conclusions: In trials of BM stimulation with G-CSF for the treatment of CVD, the prevalence of diabetes is the major negative determinant of CD34+ cell mobilization. These data strongly support that diabetes impairs stem cell mobilization, with possible negative implications for CVD.

1. Introduction

The bone marrow (BM) harbors regenerative cells involved in the homeostasis of the cardiovascular system, including endothelial (EPCs), smooth muscle and cardiomyocyte progenitors [1]. These progenitor cell lineages derive from the common immature CD34+ stem cell population within the BM. In steady-state conditions, CD34+ cells circulate at a very low frequency (<0.01% of white blood cells) in peripheral blood, but can be mobilized from the BM by different stimuli, either physiologic or pathologic [2]. Vascular and myocardial injury, through the release of chemokines and growth factors, potently stimulates the BM to mobilize CD34+ cells into the bloodstream, as a reparative attempt [3]. Preclinical studies indicate that CD34+ cells and EPCs repair the damaged vasculature, promote angiogenesis and favor recovery of the infarcted myocardium [4].

On this background, several clinical trials have been conducted during the last 10 years using autologous stem/progenitor cells to treat cardiovascular disease (CVD) [5]. Cellular products were obtained by either BM aspiration or apheresis of peripheral blood mobilized with granulocyte-colony stimulation factor (G-CSF). Other trials were designed to test whether simply increasing circulating stem cell levels with G-CSF improved patients' outcomes [6].

Meanwhile, diabetic patients have been shown to be markedly deprived of CD34+ cells and EPCs [7], possibly explaining their inability to repair cardiovascular damage [1]. Indeed, the reduction and dysfunction of vascular progenitor cells in the presence of DM or other risk factors are considered a factor limiting the efficacy of autologous cell therapy protocols [8]. In addition, animal studies consistently indicate that DM negatively affects BM structure and

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function, reducing the response to mobilizing stimuli [9–12]. Ferraro et al. in a recent retrospective analysis of patients with hematological malignancies undergoing BM mobilization with chemotherapy plus G-CSF found that diabetes and hyperglycemia were negative determinants of the CD34+ cell yield [13].

In the present study, to test whether this holds true also in a setting that is more relevant to diabetic complications, we collected data on trials of G-CSF administration in patients with CVD. Given that studies do not normally report mobilization efficiency in DM versus nonDM patients, we performed a meta-regression analysis testing the correlation between CD34+ cell levels and prevalence of DM in the study cohort.

2. Materials and methods

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

2.1. Objective

The study was conducted to detect a significant correlation between prevalence of diabetes in each trial and achieved absolute CD34+ cell count (cells/μL) after G-CSF stimulation, as an indicator of mobilization (primary end-point). A separate analysis was pre-specified for trials reporting CD34+ cell levels before and after G-CSF administration. Finally, we looked for studies eventually reporting CD34+ cell levels separately for DM and nonDM patients or individual patient data.

2.2. Literature search

We searched the English literature from 1997 to 2012 (last updated 30 Aug 2012) for articles describing clinical trials in which patients with CVD were subjected to G-CSF stimulation for BM CD34+ stem cell mobilization alone or followed by apheresis and cell therapy. The search terms were: ("trial" OR "patients" OR "patient") AND ("G-CSF" OR "GCSF" OR "granulocyte colony") AND ("CD34" OR "HSC" OR "stem cell" OR "stem cells") AND ("mobilization" OR "mobilized" OR "mobilize") AND ("vascular" OR "cardiovascular" OR "myocardial" OR "cardiac" OR "critical" OR "arterial" OR "coronary artery disease" OR "angina" OR "heart failure"). We also examined cross-references among different articles. The minimal criteria for inclusion of the study in the primary analysis were: 1) description of the study cohort with clear indication of the prevalence of diabetes; 2) clear reporting of G-CSF dosage and treatment duration; 3) indication of the absolute CD34+ cell count (in cells/μL of peripheral blood) achieved after the course of G-CSF administration.

2.3. Quality assessment

Quality of the trials included in this meta-analysis was assessed according to an itemized methodological quality graph, as suggested by the Cochrane Handbook for Systematic Reviews of Interventions [14]. Each item was scored as low, uncertain, or high risk of bias. The items included in the checklist and visualized in the graph were selected based on their relevance to the meta-regression on CD34+ cell mobilization. Therefore, detection bias was not related to the primary outcome of the trial, but on integrity of CD34+ cell data. Attrition bias was not considered, as availability of CD34+ cell data was an inclusion criterion. Quality was scored by the 2 authors independently. Quality assessment was meant to generate separate analyses limited to trials with low risk of bias.

2.4. Data retrieval

We collected the following data: mean ± SD age of the patients, percentage of male patients, prevalence of diabetes, hypertension, dyslipidemia, and smoking habit, underlying disease, baseline CD34+ cell count/μL (when available), achieved CD34+ cell count/μL after the course of G-CSF stimulation. Total dose of G-CSF (μg/kg) received by the patient was calculated as daily dose (μg/kg/day) multiplied by the duration of treatment in days. The standard course of G-CSF stimulation is 5 μg/kg b.i.d. for 5 days, for a total of 50 μg/kg. As the total dosage varied considerably among studies, in order to make the achieved CD34+ cell count comparable, we normalized CD34+ cells/μL to the standard 50 μg/kg dosage using a proportionality formula: adjusted CD34+ cells/μL = reported CD34+ cells/μL × 50 ÷ total μg/kg G-CSF administered. In case the CD34+ cell count/μL was not directly reported, this was calculated by multiplying % of CD34+ cells in peripheral blood for total white blood cell count/μL. When CD34+ cell count was reported only in figures and not in the text, data were extrapolated from figures, but these data were considered low quality and marked as such.

2.5. Statistical analysis

Data are expressed as mean ± SD or as percentages, where appropriate. For merging continuous normal data from different studies (e.g. age) in order to provide a global overview of patients characteristics, we calculated the weighted average and standard deviation. Heterogeneity among studies was calculated using the chi-square test. The correlations between prevalence of diabetes and achieved CD34+ cell count or increase in CD34+ cell count were checked using the meta-regression method described by Thomson and Higgins [15], with the random effect model. Pearson's r and Spearman's rho correlation coefficients were also calculated. A multivariate analysis was run to verify whether the correlation between prevalence of diabetes and CD34+ cell count was independent from the prevalence of other risk factors and underlying disease. The PRISMA guidelines were used to compile and report the data [16]. Statistical significant was accepted at $p<0.05$.

3. Results

3.1. Search results

Based on search criteria, we initially retrieved 227 articles for screening. Of these, 96 were retained for further evaluation, while the others were discarded as nonrelevant. Twenty-four articles were finally included in the analysis of the primary end-point, with two trials reporting two groups with different G-CSF dosages, which were considered separately (Table 1). Of the 24 articles, 13 also reported the baseline CD34+ cell count, which allowed a calculation of the increase in CD34+ cells after G-CSF stimulation (Fig. 1). In one trial only, crude data of individual patients were reported [17], allowing a calculation of the achieved CD34+ cell count in DM and nonDM patients.

3.2. Data quality assessment

Data quality is represented by an itemized methodological quality graph (Fig. 2). Most studies (70%) were randomized, controlled trials almost free from baseline imbalance, in which patients were randomly assigned to treatment with G-CSF or placebo. This suggests that the risk of selection bias was low. Quality of CD34+ cell data was high in 80% of cases: in 2 trials data were calculated from % of CD34+ cells and WBC counts (uncertain risk of bias); in 3 trials, CD34+ cell levels were derived from figures (high risk of bias). Blinding was relatively low, but this was unlikely to affect the effects of G-CSF on CD34+ cells.

3.3. Study population

Pooled characteristics of the study population are reported in Table 2. On average, patients were about sixty years old, prevalently males, treated for ST-elevation myocardial infarction. In most trials, prevalence of DM was lower than that of other traditional risk factors, reflecting the typical characteristics of patients in cardiovascular trials. The total dose of G-CSF administered was close to the standard 50 μg/kg dose, over a period of about 6 days. However, there was considerable heterogeneity in patient characteristics and treatment protocol among studies. Importantly, the chi-square test for heterogeneity among studies was statistically significant ($p<0.0001$), indicating that the study differed in the degree of CD34+ cell mobilization.

3.4. Meta-regression analysis

The primary aim of the analysis was to find a correlation between prevalence of DM and achieved absolute CD34+ cell count after G-CSF administration. Simple correlation analysis revealed a strongly significant negative correlation ($r=-0.68$; $p<0.0001$; $rho=-0.65$; $p<0.0001$). Using the random effect model by Bayesian tau taking into account weights of the single trials, the negative correlation was as well highly significant ($p<0.0001$; equation $y=a+bx$ where $a=91.2\pm11.4$; $b=-1.4\pm0.3$. Fig. 3A). The analysis of variance (calculated as the residual error sums of squares and estimated using the chi squared test) was not significant ($p=0.72$), indicating no residual heterogeneity among trials. Excluding trials in which the absolute CD34+ cell count/μL was calculated or derived