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Association of pro-inflammatory cytokines and monocyte subtypes in older and younger patients on clinical outcomes after mechanical circulatory support device implantation

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Abstract

Noninvasive immunologic analysis of peripheral blood holds promise for explaining the mechanism of development of adverse clinical outcomes, and may also become a method for patient risk stratification before or after mechanical circulatory support device (MCSD) implantation. Dysregulation of the innate immune system is associated with increased patient age but has yet to be evaluated in the older patient with advanced heart failure undergoing MCSD surgery.

Patients pre- and post-MCSD implantation had peripheral blood mononuclear cells (PBMC) and serum isolated. Multiparameter flow cytometry was used to analyze markers of innate cell function, including monocyte subtypes. Multiplex cytokine analysis was performed. MELD-XI and SOFA scores were utilized as surrogate markers of outcomes.

Increased levels of pro-inflammatory cytokines including IL-15, TNF-α, and IL-10 were associated with increased MELD-XI and SOFA scores. IL-8, TNF- α, and IL-10 were associated with risk of death after MCSD implantation, even with correction for patient age. Increased frequency of ‘classical’ monocytes (CD14 + CD16−) were associated with increased MELD-XI and SOFA scores.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2018.11.004.

This suggests that inflammation and innate immune system activation contribute to progression to multiorgan system failure and death after MCSD surgery. Development of noninvasive monitoring of peripheral blood holds promise for biomarker development for candidate selection and patient risk stratification.

Keywords

Immunosenescence; Inflammation; Monocytes; Aging; Heart failure; Ventricular assist device

1. Introduction

For the growing numbers of older patients with advanced heart failure, mechanical circulatory support device implantation can be an effective intervention for patients not manageable by medical therapy alone, either as a bridge to heart transplantation or as destination therapy [1–4]. However, older patients experience increased rates of death and inability to bridge to heart transplantation compared with younger patients with similar severity of heart failure [4]. Inflammation and pro-inflammatory changes in the innate immune system are known to be associated with normal aging, and may be part of the mechanism of progression of heart failure and atherosclerosis [5,6]. It has been described that MCSD implantation leads to inflammation and innate immune changes [7–11]. Given the many deleterious effects of inflammation, sometimes termed ‘inflammaging’ in the geriatrics literature [12,13], we propose that measurement of inflammation and innate immune changes before and after implantation will provide insight into the mechanism of development of adverse outcomes after MCSD, and may provide help with patient risk stratification in combination with currently validated clinical tools such as INTERMACS score.

Our previous work has demonstrated increased frequency of immunosenescent and terminally differentiated CD8+ T cells in older patients and in patients with adverse outcomes after MCSD implantation [14]. We have additionally observed an association between clinical outcomes and changes in gene regulation in PBMC with important immune functions including T cell differentiation, KIR expression, and the TFG-beta receptor [15].

Development of additional tools for patient candidacy evaluation is especially important to consider given the observation that older and frailer patients are at increased risk for death after MCSD implantation [4,16–19]. The potential link between markers of inflammation including cytokines and monocyte subtypes and adverse outcomes after MCSD in younger and older patients has not been previously examined. Monocyte subtypes as defined by CD14 and CD16 expression include the classical (CD14++/CD16−), intermediate CD14++/CD16+), and non-classical CD14+/CD16++), of which the classical is most strongly associated with inflammation and response to innate immune system stimuli including pro-inflammatory cytokines [20,21]. We hypothesized that increased levels of pro-inflammatory cytokines and innate immune system dysregulation are associated with adverse clinical outcomes after MCSD implantation.

2. Methods

2.1. Patients and samples

We enrolled patients undergoing evaluation for MCSD from the Ronald Reagan Medical Center with advanced heart failure, as described previously [14]. This observational study was approved by the UCLA Institutional Review Board. All patients signed informed consent. Older patients were defined as those ≥age 60. Blood was collected for serum and peripheral blood mononuclear cell (PBMC) isolation within 24 h prior to MCSD implantation and on Days 1, 3, 5, 8, 10, 14 and 17, ± 1 day after surgery. 27 patients were enrolled who had PBMC available after MCSD implantation for analysis, and completed at least 6 months of clinical follow-up between September 2012 and March 2015, with the last point of clinical review as of July 1, 2016. 24 patients had PBMC available prior to implantation. Our previous studies have confirmed the reproducibility of frozen and thawed samples for immunologic testing [14,15]. The following FDA-approved durable MCSD were included in our analysis: HeartMate II, HeartWare, Thoratec paracorporeal ventricular assist device, CentriMag, or Total Artificial Heart, but not percutaneously inserted devices such as extra-corporeal membranous oxygenation, TandemHeart, or Impella pumps. PBMC were isolated using previously published techniques [22], and frozen for storage until batched analysis could be performed. The majority (75%) of samples were collected between Days 0 and 8, with 57% with 5 samples and 71% with ≥ 5 samples available for analysis.

2.2. Flow cytometry

Viable cells were identified using a fluorescent live/dead marker (Life Technologies). Innate cell subsets were evaluated using a cocktail of fluorochrome-conjugated antibodies. CD14 and CD16 were used to define monocyte subsets as previously described [23]. CD56 and KLRG1 were utilized to define NK cells, and CD284 (Toll like receptor) to define possession of antibacterial properties and ability to recognize bacterial LPS [24]. The complete set of subtypes analyzed were as follows: CD14, CD56, CD14++/CD16−, CD14++/CD16++, CD14−/CD16+, CD14-/CD16−, CD14+/CD284+, CD14+/CD16− CD284+, CD14+/CD16+/CD284+, CD14−/CD16+/CD284+, CD14−/CD16− CD284+, CD14+/CD284+, CD14−/CD284+, CD14++/CD16++ of monocytes, and CD14−/CD16 + of monocytes. Antibodies were obtained from either BD Biosciences or Biolegend. Fluorescence from viable cells was measured by the BD LSRFortessa (BD Biosciences) with analysis via FCS Express software (DeNovo Software).

2.3. Multiplex cytokine analysis

Human 38-plex magnetic cytokine/chemokine kits were purchased from EMD Millipore and used per manufacturer's instructions. The following analytes were detected: G-CSF, GM-CSF, IFN-gamma, IFN-a2, IL-1beta, IL-1Ra, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 (p40), IL-15, IL-17A, MCP-1, MIP-1alpha, MIP-1beta, CD40L, MDC, TNF-alpha, EGF, FGF-2, GRO, Eotaxin, Fractalkine, FLT-3L, and VEGF. Fluorescence was quantified using a Luminex 200™ instrument. Cytokine values measured in pg/ml.

2.4. Clinical data collection

Prospectively collected data was used to calculate MELD-XI (Model for End-Stage Liver Disease eXcluding INR) and SOFA (Sequential Organ Failure Assessment) scores, used as a surrogate for multiorgan dysfunction [25,26]. For MELD-XI calculation, an appropriate measure for patients on anticoagulation, serum creatinine was set to 1.0 for patients with creatinine levels of < 1.0 to prevent calculation of negative numbers, generating a minimum score of 9.44, following previously published guidelines, and as described previously [14,26]. Records were reviewed for 3 month prior to and 6 months after MCSD implantation for evidence of infection, including sepsis syndrome, bacteremia, driveline infection, pneumonia, and urinary tract infection following standard definitions [27,28]. Severe infection was defined as requiring intravenous antibiotic treatment and/or leading to extension of hospital stay or death. Information on bypass time and peri-operative blood transfusion was not available for 3 patients (2 older and 1 younger). Median values were used to divide patients into “High” or “Low” MELD-XI and SOFA groups either prior to or after MCSD implantation. The median value and High/Low cutoff prior to MCSD implantation was 18; after MCSD implantation the median value and High/Low cutoff was 16. The median follow-up time was 474 days (IQR 91 to 779 days).

2.5. Statistical analysis

Individual comparisons between immune subtypes were performed as a screening analysis using JMP Pro 11 (SAS Software). Differences between continuous values (SOFA or MELD-XI, chronologic age, frequencies of immunologic subtypes) were compared by nonparametric 2-sample test (Mann-Whitney U-Test), while differences between categorical variables were compared by Fisher exact test. Standard least squares regression was used to compare numeric variables.

To correct for the issue of repeated measures and patient-to-patient variability, linear mixed effects models were used to evaluate the association between immune phenotype and clinical outcomes utilizing MELD-XI and SOFA scores, including random patient effects. Unadjusted associations between immune phenotypes and clinical outcomes were evaluated to identify candidate variables for inclusion into the multivariable regression models. Among those which were statistically significant, subsets were selected to limit the number of variables in each model based on a priori plausible clinical pathways [6,12,13]. Time to infection and death were analyzed using Cox proportional hazards models, with immune phenotypes included as time-varying covariates. Two models were constructed for data analysis: a static regression model, using actual variable values, and a dynamic predictor model, modeling each variable relative to its value at time 0 (MCSD implant). These analyses were performed using R v 3.3.2 (http://www.r-project.org/).

3. Results

3.1. Patient characteristics at time of MCSD implantation

Twenty-seven patients with advanced heart failure underwent testing enrolled in as described above (Table 1A). Patient age ranged from 25 to 81 years old. Primary indication for implantation was nonischemic heart failure and the primary device utilized was HeartMate

II. Median bypass time was 111 min in younger patients and 88.5 in older patients (p = 0.273). The number of PBMC transfused was 4 units in younger patients and 6 units in older patients (p = 0.371). Patients were divided into high (≥18) and low (< 18) MELD-XI group at the time of implant. Older patients (median age 67) were less likely to have nonischemic heart disease as the cause of heart failure, and as previously noted, older patients were more likely to have higher MELD-XI and SOFA scores at the time of MCSD implantation compared with younger patients (median age 42) [14] (Table 1B). INTERMACS scores tended to be more severe in older compared with younger patients, although this did not reach statistical significance.

3.2. Patient outcomes after MCSD implantation

Infectious complications were observed both prior to and after MCSD implantation (Table 2A). These included bacterial infections such as pneumonia and sepsis, candida infection, and viral pneumonia. 22.2% of all patients (n = 6) died by 3 months after implant. Of the 9 patients who died by one year post implant, the majority (n = 9) died due to multiorgan failure, often with sepsis. We observed a statistically significant association between older patient age and death 3 months after MCSD implantation (p = 0.020). Older patients demonstrated increased incidence of infection and severe infection in this cohort (Table 2B). There was no significant difference in bypass time in patients with or without severe infection (107 versus 101 min, p = 0.977), or in PBMC transfused (5 versus 6 units, p = 0.975).

3.3. Monocyte characteristics and patient age

Older patients demonstrated a trend towards increased frequency of classical CD14++CD16- monocytes prior to MCSD implant as compared with younger patients, with a median frequency of 31.5% as compared with 14.0% (p = 0.224). There were no statistically significant differences prior to MCSD implant for intermediate CD14 + CD16 + monocytes (2.2% compared with 2.0%, p = 0.385) or non-classical CD14−CD16++ monocytes (2.0% compared with 2.2%, p = 0.505) between older and younger patients, respectively.

After MCSD implantation (excluding pre-implant values), differences were observed in monocyte subtypes between older and younger patients, with increased frequency of classical CD14++CD16− monocytes as compared with younger patients, with a median frequency of 28.7% as compared with 15.5% (p = 0.001) (Fig. 1). There were also statistically significant differences observed for intermediate CD14 + CD16 + monocytes (2.5% compared with 0.91%, p = 0.010) and nonclassical CD14-CD16++ monocytes (1.9% compared with 1.2%, p = 0.009) between older and younger patients, respectively. Median values for older and younger patients are shown in Supplementary Tables 1A and 1B.

Differences in monocyte subtypes did not vary by INTERMACS score, measured either before or after MCSD implantation (data not shown).

3.4. TLR4+ expression and patient age

We also noted differences in TLR4+ (CD284) expression, a surface molecule important in innate immune function, which binds bacterial LPS. Older patients demonstrated decreased

frequency of monocytes expressing this surface protein, with median frequency of TLR4 + expression of 5.3% for older patients compared with 48.6% in younger patients after MCSD implantation ($p < 0.001$) (Fig. 2A). This observation was interesting given that older patients displayed an increased percentage of total monocytes (48.6% compared with 29.1% in younger patients, $p < 0.001$) (Fig. 2A).

Repeating this analysis by monocyte subtypes revealed similar results, with older patients demonstrating decreased frequency of TLR4 + expression in classical (2.4% compared with 48.6% in younger patients, $p < 0.001$), intermediate (33.0% compared with 86.8% in younger patients, $p < 0.001$), and nonclassical monocytes (14.8% compared with 54.0% in younger patients, $p < 0.001$) (Fig. 2B). Median values for older and younger patients are shown in Supplementary Tables 1A and 1B.

3.5. Plasma cytokines and patient age

Analysis of cytokine expression after MCSD implantation revealed multiple pro-inflammatory cytokines associated with the older patient. These include TNF-α ($p < 0.001$), IL-10 ($p = 0.002$), IL-15 ($p < 0.001$), TGF-α ($p = 0.013$), IL-8 ($p < 0.001$), MDC ($p = 0.006$), IP-10 ($p = 0.010$), and MCP-1 ($p < 0.001$) (Table 3). Increased levels of many of the pro-inflammatory cytokines were seen across all older patients regardless of progression to severe infection. However, we found that MCP-1 was uniquely elevated only in older patients with severe infection (median 638), while for older patients without severe infection, it was significantly lower, similar to levels seen in younger patients (median 465) ($p = 0.024$). Similarly, there were several cytokines that were significantly elevated in younger patients with severe infection but lower in younger patients without severe infection including TNF-a ($p < 0.001$), IL-8 ($p = 0.009$), and IP-10 ($p = 0.018$). These cytokines are also analyzed under Multivariate Analysis below demonstrating the independent impact of age as well as levels of proinflammatory cytokines in predicting outcomes after MCSD implantation (Tables 4 and 5).

3.6. Innate immunity and MELD-XI/SOFA score

After MCSD implantation, an association between MELD-XI score and monocyte phenotype was observed: Categorizing each MELD-XI score as ‘high’ (≥16) or ‘low’ (< 16) (divided by level post-implant) revealed higher frequencies of classical CD14++CD16− monocytes with high MELD-XI (31.4%) as compared with low MELD-XI (19.4%) ($p < 0.001$) (Fig. 2). There was also an increased frequency of intermediate CD14 + CD16 + monocytes with high MELD-XI (3.2%) compared with low MELD-XI (1.1%) ($p < 0.001$) and nonclassical CD14-CD16 + monocytes (2.0% compared with 1.6%, $p = 0.036$) (Fig. 2).

Similarly, higher frequencies of classical CD14++CD16− monocytes were observed with high SOFA (29.2%) as compared with low SOFA scores (< 6) (19.7%) ($p = 0.006$). There was also an increased frequency of intermediate CD14 + CD16 + monocytes with high SOFA (2.6%) compared with low SOFA (1.1%) ($p = 0.012$). However, there was no significant difference between nonclassical CD14-CD16 + monocytes and SOFA score (1.8% compared with 2.0%, $p = 0.686$).

Analysis of TLR4 + expression on monocytes did not demonstrate an association between MELD-XI score, although there was an increased frequency of monocytes observed in the high MELD-XI group (48.6% compared with 34.5%, p = 0.010). (Fig. 3). A similar pattern was observed for SOFA score, with no association by TLR + expression but an association with high SOFA score and increased frequency of monocytes (47.7% versus 35.4%, p = 0.007). There was however, no significant association between MELD-XI or SOFA score in non-monocyte TLR4 + cells (data not shown).

3.7. Innate immunity and clinical outcomes

Frequency of monocyte subtypes was not significantly associated with infection after MCSD implantation (data not shown). Using this nonparametric analysis approach, there was similarly no observed association between monocyte subtypes and death or successful bridge to transplant after MCSD implantation (data not shown).

For TLR4+ expression, a significant association was observed between infection and decreased expression of TLR4 on monocytes, with 6.5% in those with infection compared with 34.4% in those without (p = 0.012). This association was also seen for non-monocyte cells, with 4.5% frequency of CD14−TLR4+ cells in those with infection compared with 29.4% in those without (p = 0.005). In contrast, the expression of total monocytes was not different between groups (45.5% compared with 42.3%, p = 0.818). An association was also observed between TLR4+ monocytes subtypes, with patients with infection demonstrating decreased frequency of TLR4+ expression in classical (5.0% compared with 37.8%, p = 0.009), intermediate (37.2% compared with 88.8%, p = 0.008), and nonclassical monocytes (18.1% compared with 53.8%, p = 0.024) compared with patients without infection.

However, no association was observed between frequency of total monocytes or monocytes expressing TLR4+ and death or successful bridge to transplant by nonparametric analysis (data not shown).

3.8. Monocyte subtypes and T cell immunosenescence

Our previous studies demonstrated an association between T cell immunosenescence and adverse clinical outcomes after MCSD implantation [14]. We found that the intermediate monocyte subtype CD14 + CD16+ was significantly associated with CD8+ T cell immunosenescent or exhaustion subtypes including KLRG1+ (p = 0.002), KLRG1+/CD38+ (p < 0.001), and KLRG1+/PD1+ (p = 0.006). There was no association, however, between CD8+ T cell immunosenescence or exhaustion and expression of TLR4 (data not shown.)

3.9. Multivariable analysis combining demographic, innate immunity

To address the issue of repeated measures and individual patient effects on the association between immune phenotype and clinical outcomes, a linear mixed effects model was used. This allows for correction for random effects at the individual patient level and provides flexibility to model varying slopes and intercepts, providing an ideal analysis approach for sequential observations across a group of patients. In addition, we used this approach to assess the predictive impact of multiplex cytokine analysis, which are less amenable to individual analysis due to issues with repeated measures. Multivariable mixed effect models

predicting MELD-XI score revealed a statistically significant association between one pro-inflammatory cytokine, IL-15, ($p = 0.005$) and the pro-inflammatory classical monocyte subtype ($p = 0.001$) (Table 3). Interestingly, for SOFA score, the classical monocyte subtype was again significantly associated ($p = 0.001$), but two different pro-inflammatory cytokines were identified, IL-8 ($p < 0.001$) and MCP-1 ($p = 0.015$), although the effect size for MCP-1 was relatively small (Table 3).

Multivariable analysis of time to infection after MCSD implantation did not identify any significant associations, although in unadjusted analysis a trend was observed for age and the EGF and MDC cytokines. For prediction of time to death, the TLR4+ expressing non-monocytes population demonstrated a statistically significant association ($p = 0.023$), while a trend towards association was observed for age and the TNF-α and IL-8 cytokines (Table 3).

In order to analyze how the change in levels of the immunologic predictors predicts the change in the level of the outcomes measured, we additionally performed a dynamic analysis using a similar mixed model approach to correct for repeated measurements. Analyses were repeated with values expressed as change from baseline to attempt to better capture the dynamic nature of changes in the innate immune system and cytokine expression after MCSD implantation. Dynamic multivariable mixed effect models predicting MELD-XI score revealed a statistically significant association between change in IL-8, over time ($p < 0.001$) and trend towards significance for change in GCSF ($p = 0.067$) and IL-5 ($p = 0.069$) (Table 4). Interestingly, for SOFA score, change of the classical monocyte subtype over time was significantly associated with change in SOFA score ($p = 0.021$) as seen in the static models (Table 4). There were also several pro-inflammatory cytokines that were identified in the dynamic model: IL-6 ($p = 0.019$), IL-15 ($p = 0.021$), and MDC ($p = 0.027$) (Table 4). A trend towards statistical significance was also seen for IL-8 ($p = 0.098$).

4. Discussion

Inflammation is know to play a role in adverse outcomes in older patients, however, less is known about the role of pro-inflammatory monocytes and cytokines in the setting of MCSD implantation. Previously assessed markers such as C-reactive protein and procalcitonin have not added significantly to prediction of adverse outcomes after MCSD, suggesting the need for more immunologically oriented assessment [29,30]. In this analysis of MCSD recipients, we found that despite similar etiologies of heart failure and types of interventions, older patients demonstrated increased frequency of the pro-inflammatory CD14++/CD16− classical monocyte. In contrast, older patients had decreased frequency of TLR4+ monocytes compared with younger patients, which may explain the mechanism of increased frequency of sepsis and death in older patients after MCSD implantation. Increased frequency of the pro-inflammatory CD14++/CD16− classical monocyte was also correlated with clinical outcomes as measured by MELD-XI and SOFA scores. Mixed effect analysis correcting for repeated measures further supported the association between inflammation and adverse clinical outcomes, with statistically significant association between monocyte subtype and expression of pro-inflammatory cytokines (Table 3). Repeating this analysis as dynamic change in immune phenotype and cytokines revealed a similar pattern of

association between inflammation and adverse clinical outcomes as represented by increased MELD-XI and SOFA scores (Table 4). This dynamic analysis suggests that it is not just pre-implant inflammation related to advanced heart failure that leads to adverse outcomes, or the immediate post-surgical stress, but rather that ongoing or worsening inflammation occurring after MCSD implantation drives adverse outcomes as measured by increased MELD-XI and SOFA scores, and ultimately leading to multiorgan system failure and patient death.

These findings demonstrate that patient age is associated with differences in innate immune phenotype and cytokine expression, revealing a mechanism for the increased rates of adverse outcomes including sepsis and multiorgan dysfunction observed in older patients. This immune profile is an important predictor of adverse outcomes independent of patient age in both the static and dynamic models (Tables 3 and 4), suggesting the potential benefit of immune profiling in conjunction with currently utilized methods of patient risk profiling such as INTERMACS score, as suggested in our previous analysis of T cell immune senescence and change in gene expression and clinical outcomes after MCSD implantation [14,15]. Although there is necessarily some overlap between innate immune system changes seen in all older patients and those seen in patients who experience adverse clinical outcomes, our analyses demonstrate that several cytokines such as IL-8 and MCP-1 may possess the ability to discriminate between patients with typical age-associated immunologic changes and those at increased risk for infection or death. Validation of this approach could lead to a model of noninvasive testing of patients with advanced heart failure to predict outcomes after MCSD placement and allow for noninvasive post-operative monitoring of patients after device implantation in both biologically and chronologically older patients.

Our previous work demonstrated a significant association between immunosenescence and increased patient age as well as adverse clinical outcomes including increased MELD-XI and SOFA scores, infection, and death [31]. Interestingly we found an association between immunosenescence and the intermediate monocyte subtype (CD14+/CD16+), suggesting a connection between the adaptive and innate immune system that may have implications both for both understanding mechanism of immune dysfunction after MCSD and to increase the number of markers available for patient risk stratification as described above.

This pilot study demonstrates the promise of immunologic assessment in patients undergoing MCSD implantation. Study limitations include the relatively small sample size and diversity of devices utilized. In addition, as there were relatively few patients experiencing death during the period of study observation, we were not able to establish a strong association between markers of inflammation or lack of TLR4 expression and death. The large overlap between older patients and those experiencing infectious complications makes it difficult to separate out the potentially differing impacts of these two clinical variables in a relatively small cohort, especially given that both increased age and infection favor a pro-inflammatory immunologic milieu. However, we did observe that younger patients who experienced infection demonstrated patterns of cytokine expression similar to that of older patients, suggesting the utility of this approach in identifying at risk patients. In addition, the number of patients included is similar to other studies of immune dysfunction after MCSD implantation [7,8,10]. As a single center study, we are able to include patients receiving similar medical care with similar waiting time for transplantation. In addition, our

work confirms previous studies that have also identified IL-6, IL-8, TNF-a, and MDC as associated with heart failure and MCSD implantation [6,29,32,33]. Future multicenter studies will be important to validate our pilot findings and evaluate whether pre-implant as well as post-implant assessment is associated with clinical outcomes.

For the growing numbers of older patients with advanced heart disease, MCSD remains an important intervention for either destination therapy or as a bridge to heart transplantation. Our finding that older patients demonstrate increased proinflammatory monocyte subtypes and decreased expression of TLR4, an important surface marker of ability to control bacterial infections suggests a possible mechanism for the increased frequency of adverse outcomes in older MCSD recipients. In addition, the association between proinflammatory monocyte subtypes and proinflammatory cytokine expression in patients with increases in the clinical markers MELD-XI and SOFA, suggests that increased inflammation is associated with adverse clinical outcomes including multiorgan immune dysfunction and death after MCSD implantation. Noninvasive assessment of innate immune phenotype and cytokine expression may prove to be an important tool for patient risk stratification and identification of risk factors for progression to adverse clinical complications in older patients with advanced heart disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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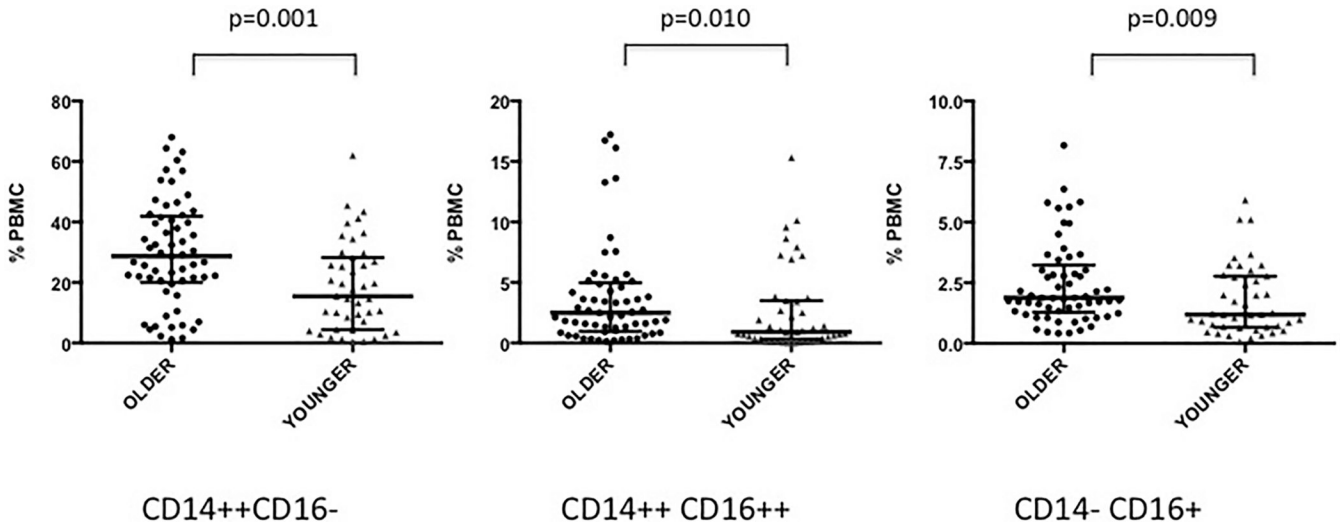

Fig. 1.

Frequency of monocyte subtypes by patient age. PBMC from time points after MCSD implantation were analyzed for classical (CD14++/CD16−), intermediate (CD14+/CD16+), and nonclassical (CD14−/CD16+) monocytes, expressed as a percentage of the total number of PBMC. Each dot or triangle corresponds to a sample; bars indicate median. p-values as indicated by nonparametric testing.

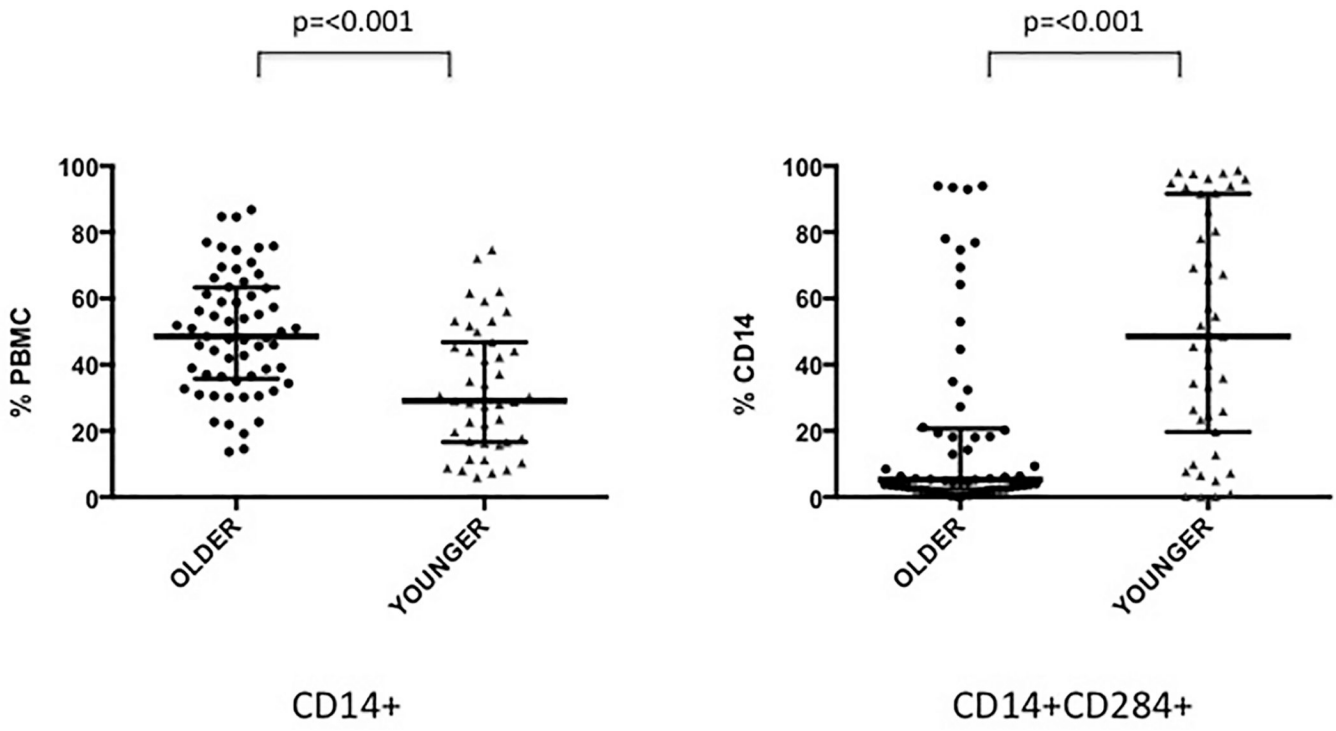

Fig. 2A.

Frequency of CD14+ and CD14 + CD284+ (TLR4+) monocytes by patient age. PBMC from time points after MCSD implantation were analyzed, expressed as a percentage of the total number of PBMC or CD14 + cells, as indicated. Each dot or triangle corresponds to a sample; bars indicate median. p-values as indicated by nonparametric testing.

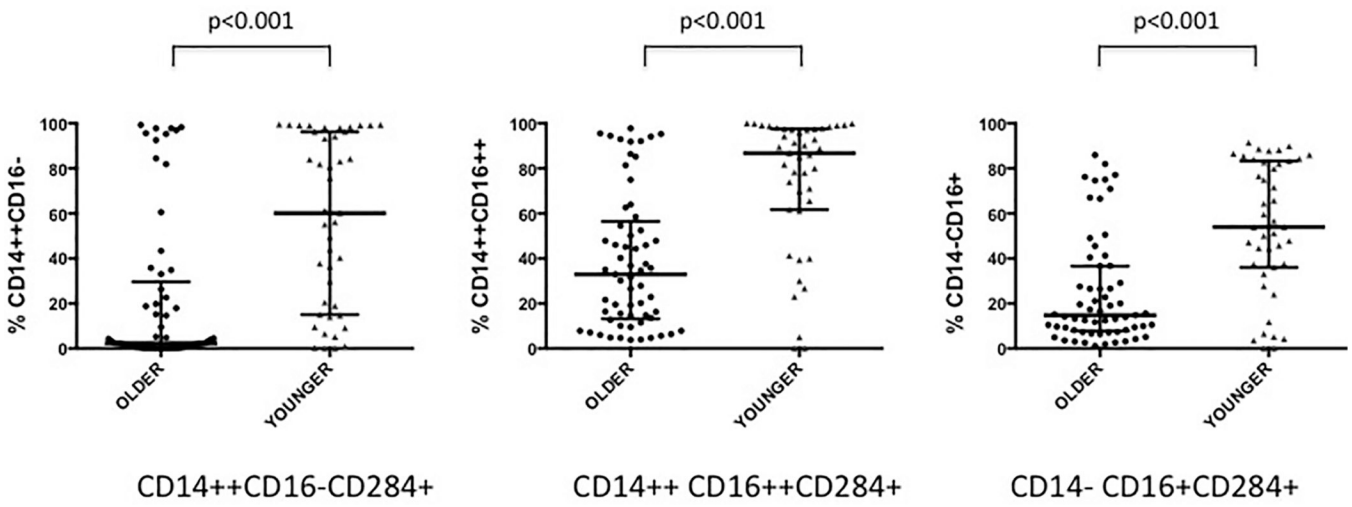

Fig. 2B.

Frequency of CD14 + CD284+ (TLR4+) monocyte subtypes by patient age. PBMC from time points after MCSD implantation were analyzed for percentage of CD284+ expression within the classical (CD14++/CD16−), intermediate (CD14+/CD16+), and nonclassical (CD14−/CD16+) monocyte subtypes. Each dot or triangle corresponds to a sample; bars indicate median. p-values as indicated by nonparametric testing.

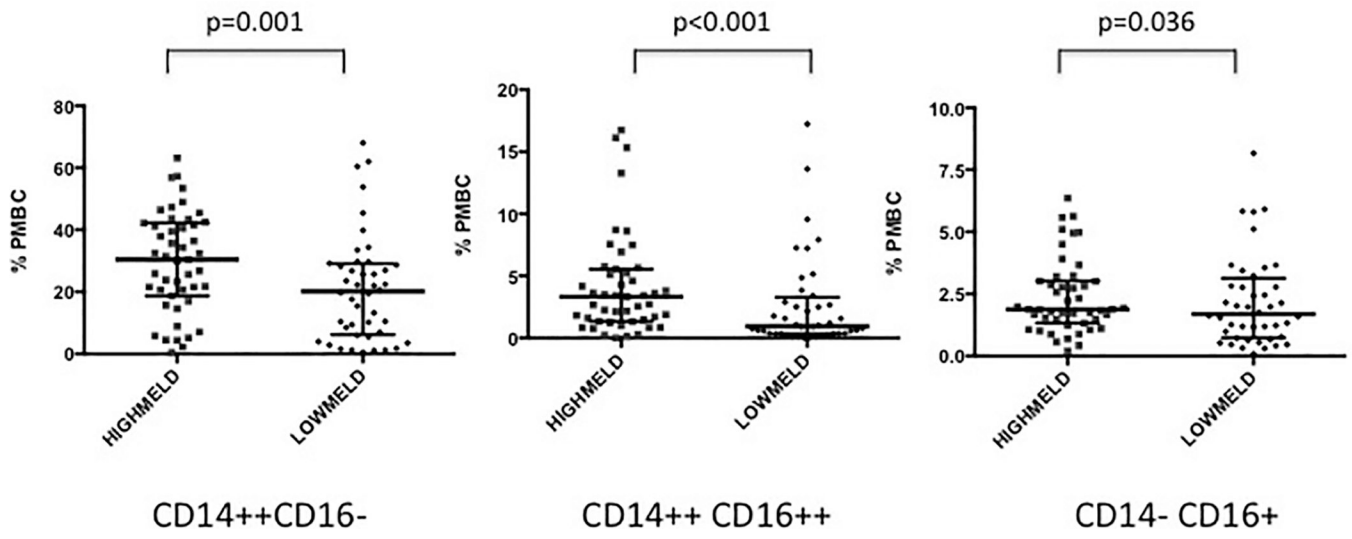

Fig. 3.

Frequency of monocyte subtypes by patient high or low MELD-XI score. PBMC from time points after MCSD implantation were analyzed for classical (CD14+ +/CD16−), intermediate (CD14+/CD16+), and nonclassical (CD14−/CD16+) monocytes, expressed as a percentage of the total number of PBMC. Each dot or triangle corresponds to a sample; bars indicate median. p-values as indicated by nonparametric testing.

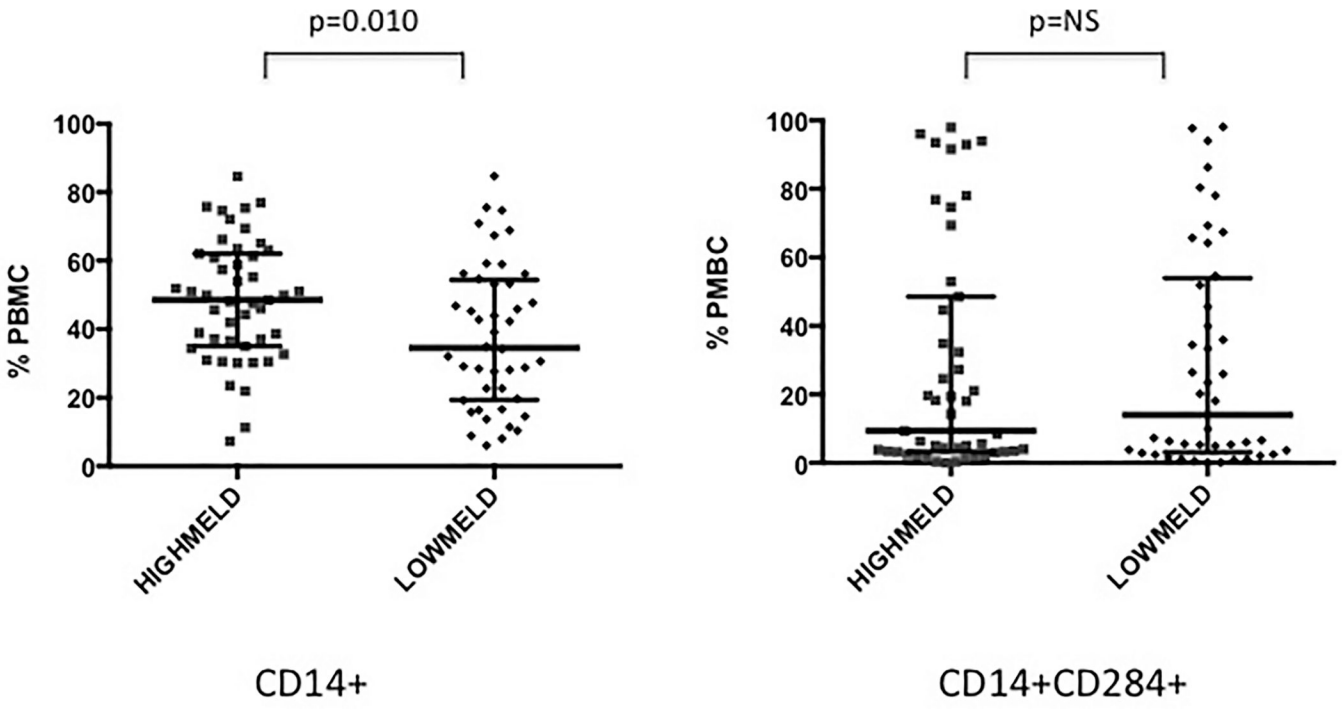

Fig. 4.

Frequency of CD14+ and CD14 + CD284+ (TLR4+) monocytes by high or low MELD-XI score. PBMC from time points after MCSD implantation were analyzed, expressed as a percentage of the total number of PBMC, as indicated. Each dot or triangle corresponds to a sample; bars indicate median. p-values as indicated by nonparametric testing.

Table 1A

Demographic characteristics of all study participants (n = 27) % (n) for each variable.

Age (yrs)(median)(range)	61 (25–81)
Older (≥age 60)	55.6% (15)
Sex (% male)	81.5% (22)
Nonischemic CMY	70.4% (19)
Intended bridge to transplantation	85.2% (23)
HeartMate II device	70.4% (20)
RVAD *	33.3% (9)
INTERMACS 1/2	59.3% (16)
MELD-XI at Day 0, median (range)	17.1 (9.4–28.8)
SOFA at Day 0, median (range)	7 (3–16)
Creatinine, mg/dl, median (range)	1.7 (1–3.7)
Total bilirubin, mg/dl, median (range)	1.6 (1–6.6)
Mean arterial pressure, median (range)	81 (54–107)

* RVAD includes right-sided Centrimag or ventricular support from PVAD or TAH.

Table 1B

Demographic characteristics of by Age ≥ 60 (Older) (n = 15) compared with patients < 60 (Younger) (n = 12) at time of implantation.

Characteristic	Older	Younger	p-value
Age (yrs) (median) (range)	67.0 (61–81)	42.0 (26–59)	N/A *
Sex (% male)	93.3%	66.7%	0.139
Nonischemic CMY	53.3%	92.7%	0.043
Intended bridge to transplantation	58.3%	83.3%	0.371
HeartMate II device	80.0%	66.7%	0.327
RVAD *	20.0%	50.0%	0.127
INTERMACS 1/2	73.3%	26.7%	0.130
MELD-XI at Day 0, median (range)	20.1 (13.0–28.8)	13.7 (9.4–21.5)	0.006
SOFA at Day 0, median (range)	8 (4–16)	5.5 (3–13)	0.047
Creatinine, mg/dl, median (range)	2.0	1.4	0.008
Total bilirubin, mg/dl, median (range)	2.0	1.5	0.111
Mean arterial pressure, median (range)	81 (54–107)	081 (69–95)	0.864

* comparison not performed as attribute used to define groups.

Table 2A

Clinical outcomes of study participants (n = 27) % (n) for each variable.

Infection pre- or post-implantation	77.8% (21)
Severe infection post-implantation	63.0% (17)
Successful bridge to transplant *	73.9% (17) *
Death 30 days	11.1% (3)
Death 3 months	22.2% (6)
Death 1 year	33.3% (9)
Days to death (median) (range)	51 (18–736)
Infection post-MCSD (≤6 months)	61.5% (16)

* Excluding 4 patients with MCS implant as destination therapy.

Table 2B

Clinical outcomes of study participants (n = 27) by Age ≥ 60 (Older) (n = 15) compared with patients < 60 (Younger) (n = 12) after implantation % (n) for each variable.

Characteristic	Older	Younger	p-value
Infection pre- or post-implantation	86.9%	60.5%	0.003
Severe infection post-implantation	75.4%	44.2%	0.002
Successful bridge to transplant *	58.3%	90.9%	0.156
Death 30 days	18.0%	0.0%	0.002
Death 3 months	39.3%	0.0%	< 0.001
Death 1 year	39.3%	25.6%	0.206
Days to death (median) (range)	32 (18–77)	137 (134–736)	0.037
Infection post-MCSD (≤6 months)	60.0% (9)	58.3% (7)	NS

* Excluding 4 patients with MCS implant as destination therapy.

Table 3

Median cytokine values in older (n = 15) as compared with younger patients (n = 12) after MCSD implantation. P values < 0.05 in bold.

Cytokine	Median (young)	Median (old)	p-value
IL-12(p40)	13.3	9.4	0.201
IL-12(p70)	11.9	13.5	0.855
IFN-g	67.5	51.2	0.857
TNF-a	20.8	31.1	< **0.001**
TNF-b	5.8	12.5	**0.042**
IL-4	3.5	6.7	**0.041**
IL-5	2.7	3.4	0.103
IL-9	2.9	3.0	**0.018**
IL-10	16.6	28.2	**0.002**
IL-13	2.4	4.1	**0.041**
IL-17A	15.3	12.7	0.564
IL-1a	8.2	23.4	0.062
IL-1b	2.7	2.7	0.445
IL-2	2.9	2.4	0.853
IL-3	3.0	3.0	0.831
IL-6	36.9	58.2	0.054
IL-15	4.8	10.3	< **0.001**
TGF-a	4.5	6.1	**0.013**
IFN-a2	41.8	46.4	0.686
IL-8	39.9	61.8	< **0.001**
GRO	339.6	334.0	0.596
Eotaxin	120.8	170.5	< **0.001**
MDC	579.0	436.5	**0.006**
IP-10	605.0	809.5	**0.010**
MCP-1	380.8	587.0	< **0.001**
MCP-3	21.0	25.6	0.147
Fractalkine	78.0	90.9	0.593
MIP-1a	6.3	8.2	0.458
MIP-1b	32.1	44.9	< **0.001**
GM-CSF	16.4	20.5	0.083
IL-7	5.3	5.9	0.493
G-CSF	61.9	76.8	0.135
VEGF	266.0	242.0	0.365
EGF	32.2	26.5	0.493
FGF-2	99.9	122.0	0.098
Flt-3L	2.5	2.5	0.399
IL-1RA	94.0	124.5	**0.014**
sCD40L	1113.0	1279.5	0.339

Table 4

Static regression models. Only variables with $p < 0.05$ in preliminary analyses included.

	MELD-XI		SOFA		Time to infection		Time to death	
Effect	**Diff. (95% CI)**	**p**	**Diff. (95% CI)**	**p**	**Diff. (95% CI)**	**p**	**Diff. (95% CI)**	**p**
Age	0.08 (−0.02–0.19)	0.120	0.01 (−0.04–0.05)	0.809	0.99 (0.96–1.02)	0.564	1.02 (0.97–1.08)	0.423
EGF	ND		ND		0.99 (0.96–1.01)	0.200	ND	
TNF-α	0.03 (−0.01–0.08)	0.120	ND		ND		1.02 (0.98–1.07)	0.383
IL-8	ND		0.03 (0.02–0.04)	< **0.001**	ND		1.01 (1.00–1.03)	0.128
IL-10	0.00 (0.00–0.01)	0.085	0.00 (0.00–0.01)	0.176	ND		ND	
IL-15	0.26 (0.08–0.44)	**0.005**	ND		ND		ND	
MCP-1	ND		0.00 (0.00–0.00)	**0.015**	ND		ND	
MDC	ND		ND		1.00 (1.00–1.00)	0.223	ND	
GCSF	0.00 (−0.01–0.01)	0.530	ND		ND		ND	
CD14 + CD16− (classical monocytes)	0.08 (0.03–0.13)	**0.001**	0.06 (0.02–0.09)	**0.001**	ND		ND	
CD14-CD284 +	ND		ND		ND		1.03 (1.00–1.06)	**0.023**

Diff.: Difference; ND, not done.

Table 5

Fully dynamic models based on variable change from baseline. Only variables with $p < 0.05$ in preliminary analyses included.

	MELD-XI		SOFA	
Effect	**Diff. (95% CI)**	**p**	**Diff. (95% CI)**	**p**
Age	0.03 (−0.12–0.17)	0.712	0.01 (−0.10–0.12)	0.865
EGF	0.04 (−0.01–0.10)	0.123	ND	
TGF	ND		−0.14 (−0.32–0.03)	0.111
TNF-α	−0.07 (−0.18–0.04)	0.209	−0.02 (−0.11–0.07)	0.628
IL-1 α	−0.01 (−0.03–0.00)	0.154	ND	
IL-5	0.16 (−0.01–0.34)	0.069	ND	
IL-6	−0.01 (−0.02–0.01)	0.393	0.02 (0.00–0.03)	**0.019**
IL-8	0.07 (0.03–0.10)	< **0.001**	0.02 (0.00–0.05)	0.098
IL-10	0.00 (0.00–0.01)	0.973	0.00 (0.00–0.01)	0.440
IL-15	0.17 (−0.11–0.46)	0.239	0.26 (0.01–0.48)	**0.021**
MCP-1	ND		0.00 (0.00–0.00)	0.482
MIP-1 α	0.02 (−0.05–0.08)	0.615	0.03 (−0.02–0.08)	0.224
MIP-1β	−0.02 (−0.08–0.03)	0.395	ND	
MDC	ND		−0.01 (−0.01–0.00)	**0.027**
GCSF	−0.01 (−0.03–0.00)	0.067	0.00 (−0.19–0.16)	0.590
CD14 + CD16− (classical monocytes)	0.03 (−0.03–0.10)	0.296	0.06 (0.01–0.12)	**0.021**
CD14-CD16+ of monocytes (nonclassical monocytes)	−0.13 (−0.34–0.09)	0.247	−0.01 (−0.19–0.16)	0.877

Diff.: Difference; ND, not done.