SERAPHIN haemodynamic substudy: the effect of the dual endothelin receptor antagonist macitentan on haemodynamic parameters and NT-proBNP levels and their association with disease progression in patients with pulmonary arterial hypertension

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Aims

The effect of macitentan on haemodynamic parameters and NT-proBNP levels was evaluated in pulmonary arterial hypertension (PAH) patients in the SERAPHIN study. Association between these parameters and disease progression, assessed by the primary endpoint (time to first morbidity/mortality event), was explored.

Methods and results

Of the 742 randomized patients, 187 with right heart catheterization at baseline and month 6 participated in a haemodynamic sub-study. Prespecified endpoints included change from baseline to month 6 in cardiac index (CI), right atrial pressure (RAP), mean pulmonary arterial pressure (mPAP), pulmonary vascular resistance (PVR), mixed-venous oxygen saturation, and NT-proBNP. Exploratory analyses examined associations between CI, RAP, and NT-proBNP and disease progression using the Kaplan-Meier method and Cox regression models. Macitentan improved CI, RAP, mPAP, PVR and NT-proBNP vs. placebo at month 6. Absolute levels of CI, RAP and NT-proBNP at baseline and month 6, but not their changes, were associated with morbidity/mortality events. Patients with CI > 2.5 L/min/m², RAP < 8 mmHg, or NT-proBNP < 750 fmol/ml at month 6 had a lower risk of morbidity/mortality than those not meeting these thresholds (HR 0.49, 95% CI 0.28–0.86; HR 0.72, 95% CI 0.42–1.22; and HR 0.22, 95% CI 0.15–0.33, respectively).
For all treatment groups, baseline and month 6 values of CI, RAP, and NT-proBNP, but not their changes, were associated with morbidity/mortality events, confirming their relevance in predicting disease progression in patients with PAH. By improving those parameters, macitentan increased the likelihood of reaching threshold values associated with lower risk of morbidity/mortality.

Keywords
Haemodynamics • Prognosis • Macitentan • Pulmonary hypertension • Right ventricular function

Introduction
Pulmonary arterial hypertension (PAH) is a chronic disease characterized by progressive symptomatic and cardiopulmonary haemodynamic deterioration. Elevated pulmonary vascular resistance (PVR) and the resulting increased right ventricular (RV) afterload contribute to the maladaptive processes of RV hypertrophy and dilatation, which eventually result in RV failure and premature death. Other hallmarks of RV impairment include reduced cardiac index (CI), increased right atrial pressure (RAP), and elevated levels of N-terminal pro-brain natriuretic peptide (NT-proBNP). Recommendations for the management of PAH advocate treatment strategies targeted to improvements in RV function as assessed by CI, RAP, and NT-proBNP. The ESC/ERS Guidelines outline risk categories for these parameters based on their association with survival in observational studies. Macitentan is a dual endothelin receptor antagonist (ERA) approved for the long-term treatment of PAH. In the randomized controlled trial SERAPHIN (Study with an Endothelin Receptor Antagonist in Pulmonary arterial Hypertension to Improve clinicaL outcome), macitentan 10 mg significantly reduced the risk for the primary endpoint of time to first morbidity/mortality event by 45% (P < 0.001). The trial included a haemodynamic sub-study; a subset of the 742 enrolled patients (n = 187) underwent right heart catheterization (RHC) at baseline and at month 6. Treatment with macitentan 10 mg resulted in significant improvements in PVR and CI at month 6 compared with placebo. Here, we describe the effect of macitentan on a broader range of haemodynamic parameters and NT-proBNP. In addition, the study allowed us to evaluate in a randomized controlled trial with a prospectively defined morbidity/mortality endpoint the association between risk thresholds for CI, RAP and NT-proBNP outlined in the guidelines and disease progression.

Methods
Study population
SERAPHIN was a multicentre, randomized, double-blind, event-driven trial (NCT00660179) and is described in detail elsewhere. Eligible patients were aged > 12 years with a confirmed PAH diagnosis. Patients naive to PAH treatment or those receiving phosphodiesterase type 5 inhibitors, oral or inhaled prostanoids, calcium channel blockers, or L-arginine at stable doses for at least 3 months prior to randomization were eligible. Patients receiving intravenous/subcutaneous prostanoids or ERAs were excluded.

Study design
After a screening period of ≤ 28 days, patients were randomly assigned in a 1:1:1 ratio to receive macitentan 3 mg or macitentan 10 mg, or placebo, once daily. Patients received double-blind treatment until they experienced a primary endpoint event, or until the end of the study (when 285 events had occurred). The primary endpoint was the time from study treatment initiation to the first PAH-related morbidity event (worsening of PAH, initiation of treatment with intravenous/subcutaneous prostanoids, lung transplantation, or atrial septostomy) or death from any cause up to the end of treatment. All primary endpoint events were adjudicated by a blinded independent committee. The trial adhered to the Declaration of Helsinki and the research protocol was approved by local institutional review boards/independent ethics committees. Written informed consent was obtained from all patients.

Haemodynamic sub-study and outcomes
A haemodynamic sub-study was conducted at 44 centres in 16 countries (Supplementary material online, Table S1). Centres were selected if they regularly followed patients by RHC. Patients were eligible if their baseline RHC was assessed within 3 months before randomization. The sub-study included a second RHC performed at month 6. Pre-specified outcomes included changes from baseline to month 6 in CI, RAP, mean pulmonary arterial pressure (mPAP), PVR, and SvO2. Changes from baseline to month 6 in NT-proBNP, measured in plasma using the BNP Fragment Enzyme ImmunoAssay BI-208528 (Biomedica Medizinprodukte GmbH, Germany), was analysed in the sub-study patients and in the overall SERAPHIN population. Safety was assessed throughout.

Statistical analyses
Changes in haemodynamic parameters and NT-proBNP at month 6
Mean changes from baseline in CI, RAP, mPAP, SvO2, and NT-proBNP were summarized descriptively by treatment group. Changes in PVR were calculated as the percentage change from baseline and summarized using geometric means and two-sided 95% CLs. This methodology was prespecified to account for the high variability in baseline PVR levels and assumed a normal distribution of the log-transformed fold change from baseline to month 6. Changes from baseline to month 6 were also analysed post hoc by WHO functional class and background PAH-specific therapy at baseline. Treatment effects between macitentan 3 mg and macitentan 10 mg vs. placebo are presented as a difference in mean changes with 95% CL, using the observed data at month 6.

Associations of CI, RAP, and NT-proBNP with disease progression
We investigated the prognostic relevance of the continuous variables for CI, RAP and NT-proBNP at baseline and month 6, and the changes in these levels from baseline to month 6, on disease progression as assessed by the primary endpoint. We also analysed whether being above or below a specific CI, RAP and NT-proBNP threshold at month 6 was associated with the risk of a morbidity/mortality event. Thresholds of 2.5 L/min/m2 for CI and of 8 mmHg for RAP were applied based on the low-risk profile described in the ESC/ERS 2015 PAH guidelines. For NT-proBNP, the threshold of 300 ng/L outlined in the ESC/ERS 2015...
guidelines could not be applied because the assay in our study was not compatible with the assay used to determine the 300 ng/L threshold. We used a threshold of 750 fmol/ml, the median NT-proBNP level at month 6 among all randomized patients with available data (n = 502).

All associations were analysed using Kaplan–Meier estimates and the log-rank test. Hazard ratios (HRs) and 95% CI were primarily calculated using univariate Cox’s proportional hazard models. The baseline analyses were conducted on patients with available baseline values. The month 6 analyses were conducted on patients who had available data at baseline and month 6, with exclusion of patients who had experienced a morbidity/mortality event before month 6. All analyses were performed on observed data. All models were also adjusted for study treatment, baseline PAH-therapy and time from RHC to randomization. Consistency of the associations between the month 6 levels and disease progression across treatment groups was assessed using interaction tests (significance level 5%).

Finally, in patients with available data at baseline and at month 6, we evaluated the proportions of patients with CI > 2.5 L/min/m², RAP < 8 mmHg, or NT-proBNP < 750 fmol/ml in each treatment group (placebo, macitentan 3 mg and macitentan 10 mg). Odds ratios, 95% CI and P-values for macitentan 3 mg vs. placebo and macitentan 10 mg vs. placebo were calculated using logistic regression models, adjusted for baseline values.

Results

Patient characteristics
Of the 187 haemodynamic sub-study patients, 68 were randomized to placebo, 62 to macitentan 3 mg and 57 to macitentan 10 mg. The majority were in WHO functional class II and III, and half of the patients were on background PAH-specific therapy, mainly sildenafil (Table 1). The baseline characteristics were consistent with the overall SERAPHIN population and were comparable across treatment groups. The mean duration between RHC and study enrolment was 0.6 (±1.1 SD) months. At month 6, RHC was performed in 147 patients. Reasons for missing data are provided in Supplementary material online, Table S2, and cohorts with complete data are indicated in the footnotes of each table.

Changes in cardiopulmonary haemodynamic parameters and NT-proBNP at month 6
Cardiopulmonary haemodynamic parameters and NT-proBNP levels worsened from baseline to month 6 in placebo-treated patients and improved in patients treated with macitentan 3 mg and macitentan

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Table 1. Demographics and baseline characteristics

|                      | SERAPHIN [Pulido 2013a] Overall patients (n = 742) | SERAPHIN haemodynamic study All patients (n = 187) | Placebo (n = 68) | Macitentan 3 mg (n = 62) | Macitentan 10 mg (n = 57) |
|----------------------|---------------------------------------------------|---------------------------------------------------|------------------|-------------------------|-------------------------|
| Female sex, %        | 77                                                | 76                                                | 74               | 74                      | 81                      |
| Age, years (mean ± SD) | 46 ± 16                                           | 47 ± 16                                           | 48 ± 16          | 47 ± 16                 | 47 ± 15                 |
| Time from diagnosis, weeks (mean ± SD) | 142 ± 208                                         | 143 ± 249                                         | 138 ± 282        | 137 ± 232               | 132 ± 229               |
| Time from RHC to randomization, months (mean ± SD) | 1.8 ± 2.9a                                        | 0.6 ± 1.1                                         | 0.5 ± 0.9        | 0.6 ± 1.1               | 0.7 ± 1.3               |
| 6MWD, m (mean ± SD)  | 360 ± 100                                          | 353 ± 103                                         | 360 ± 112        | 341 ± 100               | 359 ± 94                |
| WHO FC, n (%)        |                                                   |                                                   |                  |                         |                         |
| I                    | 1 (0.1)                                            | 1 (0.5)                                           | –                | –                       | 1 (1.8)                 |
| II                   | 387 (52.4)                                         | 81 (43.3)                                         | 32 (47.1)        | 25 (40.3)               | 24 (42.1)               |
| III                  | 337 (45.6)                                         | 100 (53.5)                                        | 35 (51.5)        | 35 (56.5)               | 30 (52.6)               |
| IV                   | 14 (1.9)                                           | 5 (2.7)                                           | 1 (1.5)          | 2 (3.2)                 | 2 (3.5)                 |
| Background PAH-specific therapy, % | 64                                                | 49                                                | 50               | 55                      | 42                      |
| CI, L/min/m² (mean ± SD) | 2.4 ± 0.8                                          | 2.5 ± 0.8                                         | 2.5 ± 0.7        | 2.4 ± 0.8               | 2.6 ± 0.9               |
| RAP, mmHg (mean ± SD) | 9 ± 6                                              | 8 ± 5                                             | 8 ± 4            | 9 ± 5                   | 8 ± 6                   |
| mPAP, mmHg (mean ± SD) | 54 ± 18                                            | 53 ± 18                                           | 53 ± 20          | 53 ± 18                 | 54 ± 17                 |
| PVR, dyn-sec/cm⁵ (mean ± SD) | 1026 ± 696.7                                     | 919 ± 548.6                                       | 900 ± 556.3      | 934 ± 564.0             | 924 ± 531.5             |
| SvO₂, % (mean ± SD)  | 65 ± 10a                                           | 65 ± 10                                           | 65 ± 8           | 63 ± 10                 | 65 ± 11                 |
| NT-proBNP, fmol/ml (mean ± SD) | n = 501                                            | n = 142                                           | n = 50           | n = 46                  | n = 46                  |
| 1070 ± 825²          | 1294 ± 960                                         | 1264 ± 1001                                      | 1447 ± 1055      | 1173 ± 800              |

In the haemodynamic sub-study, baseline RAP, mPAP, and CI values were missing for 1 patient in the placebo group; baseline PVR was missing for 3 patients (1 in each treatment group); baseline SvO₂ was missing in 15 patients (5 in the placebo group, 7 in the macitentan 3 mg group and 3 in the macitentan 10 mg group).

²Time from RHC to randomization, SvO₂ and NT-proBNP not presented in the Pulido et al. publication.

1Patient in WHO FC I was incorrectly included in the study.

²Phosphodiesterase-5 inhibitors and oral/inhaled prostacyclin therapy.

6MWD, 6-min walk distance; CI, cardiac index; mPAP, mean pulmonary arterial pressure; RAP, right atrial pressure; NT-proBNP, N-terminal pro-brain natriuretic peptide; PAH, pulmonary arterial hypertension; PVR, pulmonary vascular resistance; RHC, right heart catheterization; SD, standard deviation; SvO₂, mixed venous oxygen saturation; WHO FC, World Health Organization functional class.
Table 2

| Treatment          | n   | Mean change from baseline (95% CI) | Mean treatment effect vs. placebo (95% CI) |
|--------------------|-----|------------------------------------|-------------------------------------------|
| Macitentan 10 mg   | 48  | 0.30 ± 0.85                        | 0.69 (0.44, 0.93)*                        |
| Macitentan 3 mg    | 49  | 0.36 ± 0.57                        | 0.63 (0.43, 0.83)*                        |

CI, cardiac index; CL, confidence limits; mPAP, mean pulmonary arterial pressure; RAP, right atrial pressure; NT-proBNP, N-terminal pro-brain natriuretic peptide; PVR, pulmonary vascular resistance; SD, standard deviation; SvO2, mixed venous oxygen saturation.

associations of CI, RAP, and NT-proBNP with disease progression

CI, RAP, and NT-proBNP at baseline and at month 6

CI, RAP and NT-proBNP at baseline and at month 6 were associated with the risk of a morbidity/mortality event. For every 0.5 L/min/m² increase in CI at baseline there was a 24% lower risk (HR 0.76, 95% CI 0.70–0.83) and at month 6 a 25% lower risk (HR 0.75, 95% CI 0.62–0.89) of experiencing a morbidity/mortality event. For every 1 mmHg increase in RAP at both baseline and month 6, there was a 6% greater risk (HR 1.06, 95% CI 1.04–1.08 and HR 1.06, 95% CI 1.04–1.11) of experiencing a morbidity/mortality event. For every 300 fmol/ml increase in NT-proBNP at baseline there was a 15% lower risk (HR 0.85, 95% CI 0.79–0.91) and at month 6 a 19% greater risk (HR 1.19, 95% CI 1.15–1.24) of experiencing a morbidity/mortality event. Similar results were obtained when adjusted for study treatment, background PAH-specific therapy and time from RHC to randomization (Supplementary material online, Table S5).

CI, RAP, and NT-proBNP changes from baseline to month 6

For every increase of 0.5 L/min/m² in CI from baseline to month 6, there was a non-significant 16% reduction in the risk of morbidity/mortality events [HR = 0.84, 95% CI 0.69–1.03]. Changes in RAP at month 6 were not associated with the risk of morbidity/mortality [HR = 1.01, 95% CI 0.95–1.08; for every increase of 1 mmHg from baseline to month 6]. For every increase of 300 fmol/ml in NT-proBNP from baseline to month 6, there was a 15% lower risk (HR 0.85, 95% CI 0.79–0.91) and at month 6 a 25% lower risk (HR 0.75, 95% CI 0.62–0.92) of experiencing a morbidity/mortality event. For every 0.5 L/min/m² increase in CI at baseline there was a 24% lower risk (HR 0.76, 95% CI 0.70–0.83) and at month 6 a 25% lower risk (HR 0.75, 95% CI 0.62–0.89) of experiencing a morbidity/mortality event. For every 1 mmHg increase in RAP at both baseline and month 6, there was a 6% greater risk (HR 1.06, 95% CI 1.04–1.08 and HR 1.06, 95% CI 1.04–1.11) of experiencing a morbidity/mortality event. For every 300 fmol/ml increase in NT-proBNP at baseline there was a 15% lower risk (HR 0.85, 95% CI 0.79–0.91) and at month 6 a 19% greater risk (HR 1.19, 95% CI 1.15–1.24) of morbidity/mortality. Similar results were obtained when adjusted for study treatment, background PAH-specific therapy and time from RHC to randomization (Supplementary material online, Table S4).

CI, RAP, and NT-proBNP thresholds at month 6

After 6 months, patients with a CI > 2.5 L/min/m² vs. a CI ≤ 2.5 L/min/m² had a 51% reduction in the risk of morbidity/mortality events (HR 0.49, 95% CI 0.28–0.86; Figure 1A). Patients with RAP < 8 mmHg vs. RAP ≥ 8 mmHg at month 6 had a 28% reduction in the risk of morbidity/mortality (HR 0.72, 95% CI 0.42–1.22; Figure 1B) and patients with NT-proBNP < 750 fmol/ml vs. ≥ 750 fmol/ml at month 6 had a 78% reduction in the risk of morbidity/mortality (HR 0.22, 95% CI 0.15–0.33; Figure 1C). We observed a consistent reduction in the risk of morbidity/mortality after the adjustment for study treatment, background PAH-specific therapy and time from RHC to randomization (Supplementary material online, Table S5). At month 6, patients treated with macitentan were more likely to have a CI
Table 3  Changes from baseline to month 6 for haemodynamic parameters and NT-proBNP by WHO functional class

| WHO functional class | Placebo (n = 68) | Macitentan 3 mg (n = 62) | Macitentan 10 mg (n = 57) |
|---------------------|------------------|-------------------------|--------------------------|
|                     | Mean change ± SDa | Mean treatment effect vs. placebo (95% CL)b | Mean change ± SDa | Mean treatment effect vs. placebo (95% CL)b |
| CI, L/min/m²        | -0.34 ± 0.77     | 0.69 (0.28, 1.10)        | 0.35 ± 0.80           | 0.69 (0.22, 1.15) |
| mPAP, mmHg          | 1.0 ± 6.92       | -7.0 (-11.4, -2.5)       | -7.3 ± 13.30          | -8.3 (-14.3, -2.2) |
| PVR, %              | 118.7 (102.9, 137.0) | -40.9 (-52.8, -25.9) | 65.6 (52.3, 82.4) | -44.7 (-57.0, -29.0) |
| NT-proBNP, fmol/ml  | 194.8 ± 530.9    | -313 (-634, 7.5)         | -67.3 ± 316.3        | -262 (-534, 9.5)  |

WHO functional class III/IV

| CI, L/min/m²        | -0.32 ± 0.51     | 0.68 (0.36, 1.00)        | 0.25 ± 0.89           | 0.58 (0.16, 0.99) |
| mPAP, mmHg          | 1.1 ± 8.09       | -2.9 (-7.3, 1.5)         | -3.8 ± 9.63           | -4.8 (-9.9, 0.2) |
| PVR, %              | 112.7 (96.7, 131.3) | -27.9 (-39.7, -13.8) | 76.0 (64.3, 89.7) | -32.6 (-46.0, -15.8) |
| NT-proBNP, fmol/ml  | 193.4 ± 629.5    | -389 (-768, -9.6)        | -141 ± 685.8         | -334 (-710, 41)  |

Table 4  Changes from baseline to month 6 for haemodynamic parameters and NT-proBNP by background PAH-specific therapy

| Background PAH-specific therapy | Placebo (n = 68) | Macitentan 3 mg (n = 62) | Macitentan 10 mg (n = 57) |
|---------------------------------|------------------|-------------------------|--------------------------|
|                                 | Mean change ± SDa | Mean treatment effect vs. placebo (95% CL)b | Mean change ± SDa | Mean treatment effect vs. placebo (95% CL)b |
| With background PAH-specific therapy |                   |                          |                          |
| CI, L/min/m²                   | -0.34 ± 0.52     | 0.74 (0.43, 1.04)        | 0.28 ± 0.79            | 0.61 (0.24, 0.98) |
| mPAP, mmHg                     | 1.1 ± 6.7        | -4.8 (-8.7, -0.9)        | -3.3 ± 7.9            | -4.4 (-8.5, -0.3) |
| PVR, %                         | 119.7 (105.4, 135.8) | -37.4 (-46.6, -26.7) | 75.5 (63.7, 89.6) | -36.9 (-48.5, -22.7) |
| NT-proBNP, fmol/ml             | 37.5 ± 321.9     | -283 (-590, 25)          | -228.8 ± 501         | -266 (-503, -29) |
| Without background PAH-specific therapy |                   |                          |                          |
| CI, L/min/m²                   | -0.32 ± 0.81     | 0.60 (0.15, 1.04)        | 0.32 ± 0.91           | 0.64 (0.12, 1.15) |
| mPAP, mmHg                     | 0.9 ± 8.5        | -3.8 (-9.2, 1.6)         | -7.0 ± 13.6           | -7.9 (-14.7, -1.1) |
| PVR, %                         | 110.7 (92.7, 132.1) | -27.2 (-44.1, -5.4) | 67.8 (55.2, 83.4) | -38.7 (-53.3, -19.5) |
| NT-proBNP, fmol/ml             | 402.9 ± 757.6    | -466 (-870, -61)         | -8.2 ± 582.5         | -411 (-810, -13) |

*P-value <0.05 for the comparison between macitentan and placebo.

PVR data are expressed as a percent change (%) between macitentan and placebo: (ratio of geometric means - 1) × 100. Data for all other variables are mean ± SD.

n represents the number of patients in the haemodynamic sub-study at baseline; n represents the number of patients with non-missing values for the change from baseline to month 6.

Of the 68 placebo patients, 32 were in WHO FC III and 36 were in WHO FC III/IV; of the 64 macitentan 3 mg patients, 25 were in WHO FC III and 37 were in WHO FC III/IV; of the 57 macitentan 10 mg patients, 25 were in WHO FC III and 32 were in WHO FC III/IV.

Results are based on observed data with no imputation rules applied for missing values.

CL, confidence limits; CI, cardiac index; NT-proBNP, N-terminal pro-brain natriuretic peptide; mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance; WHO FC, World Health Organization functional class.
Consistency of findings across study arms

The associations were generally consistent across study arms (placebo, macitentan 3 mg and macitentan 10 mg), with P-values for treatment interaction indicating no statistical differences (Supplementary material online, Table S6). Differences in the associations across the study arms were observed only for changes in NT-proBNP at month 6 (treatment interaction P-value = 0.0101).

Safety and tolerability

The most frequently reported adverse events in haemodynamic sub-study patients are reported in Supplementary material online, Table S7. Similar proportions of placebo-, macitentan 3 mg-, and macitentan 10 mg-treated patients experienced at least one adverse event. Macitentan 3 mg and macitentan 10 mg were associated with increased rates of headache compared with placebo. Macitentan 10 mg was associated with increased rates of viral respiratory tract infections and bronchitis compared with placebo. No adverse events related to RHC were reported.

Discussion

In the SERAPHIN sub-study, macitentan improved haemodynamic parameters and NT-proBNP levels after 6 months of treatment in patients with PAH irrespective of WHO functional class and background PAH-specific therapy (predominantly sildenafil) at baseline, whereas these parameters worsened in patients who received placebo. These data are consistent with the favourable effects of macitentan on long-term (median duration of treatment 115 weeks) morbidity/mortality in patients with PAH. In fact, haemodynamics and NT-proBNP plasma levels, which are indirect indicators of RV function, represent a major determinant of the PAH patients’ prognosis.

The prognostic relevance of baseline CI, RAP and NT-proBNP on disease progression has been confirmed for the first time in a randomized controlled trial using a prospectively defined composite primary endpoint of morbidity/mortality. This finding highlights the importance of starting PAH medications as soon as possible, before advanced changes of RV function. In addition, the prognostic relevance of CI, RAP, and NT-proBNP after 6 months of treatment provides evidence for their contribution to the overall evaluation of the treatment response.
In clinical practice, the goal of the PAH physician is to maintain or bring their patient to a low-risk profile, part of which includes achieving and/or maintaining good RV function.\(^1,2\) In this sub-study, patients who at month 6 had a CI > 2.5 L/min/m\(^2\), RAP < 8 mmHg and NT-proBNP < 750 fmol/ml experienced a lower risk of disease progression compared with patients who did not. We also observed that patients treated with macitentan 10 mg were more likely to reach these thresholds at month 6 compared with placebo. These results do not demonstrate that improving haemodynamic parameters results in improved long-term outcomes. Nevertheless, they offer an indirect confirmation of the recommendations proposed in the ESC/ERS guidelines\(^1,2\) for the initial risk stratification and evaluation of treatment response. These findings deserve to be highlighted as they may strengthen the current level of evidence for these recommendations (level C: consensus of opinion of the experts and/or small studies, retrospective studies, registries).

The low-risk profile of the ESC/ERS guidelines includes a threshold of NT-proBNP that is based on data from observational studies.\(^4,5\) An NT-proBNP threshold of 300 ng/L (equivalent to 35 fmol/ml) is considered appropriate to identify a better prognosis.\(^1,2\) This threshold was derived from NT-proBNP levels measured by the Roche Diagnostics Elecsys NT-proBNP immunoassay. In the SERAPHIN study, the Biomedica BNP Fragment Enzyme ImmunoAssay was used. The two assays detect different epitopes; the Roche assay detects larger fragments of NT-proBNP than the Biomedica assay. Because the differences between the assays are non-linear, plasma levels derived from these assays cannot be compared.\(^11\) Although the data of this study are not comparable with the NT-proBNP threshold proposed in the guidelines, they support the use of NT-proBNP for the evaluation of treatment response.

In this study, CI, RAP and NT-proBNP values, at baseline and at month 6, were associated with disease progression, while there was no consistent evidence for an association between the changes in these parameters and long-term outcomes. In previous studies, improvement or deterioration in certain indicators of RV function were sometimes but not always associated with survival.\(^16-18\) Our study suggests that an increase in NT-proBNP was indicative of disease progression. This association was observed in the placebo arm, but was less apparent in patients treated with macitentan. The inconsistency in associations observed between changes in the parameters evaluated and disease progression may be influenced by patients’ baseline condition. Patients with characteristics indicative of low risk

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**Figure 2** Proportion of patients with (A) CI > 2.5 L/min/m\(^2\) (B) RAP of < 8 mmHg or (C) NT-proBNP < 750 fmol/ml at month 6. Adjusted for values at baseline. For CI and RAP, \(n\) denotes the number of patients who participated in the haemodynamic sub-study; for NT-proBNP, \(n\) denotes all randomized patients in the SERAPHIN study. The percentages are proportions of patients with values at month 6 who reached the respective thresholds. OR, odds ratio; CI, confidence limit; CI, cardiac index; RAP, right atrial pressure; NT-proBNP, N-terminal pro-brain natriuretic peptide.
at baseline (high CI, low RAP, or low NT-proBNP) who experience small improvements with treatment may have better outcomes than patients with indicators of high risk at baseline who experience comparatively large improvements but nevertheless remain with measures indicative of poor prognosis. Theoretically, to avoid this apparent discrepancy, changes in a given parameter should not be correlated with outcome per se, but rather with the change in risk associated with that outcome between two time points (e.g. baseline and month 6). In practice, however, only the long-term outcome can be observed, and changes in risk can only be indirectly estimated.

A limitation of these analyses is that they include only a subset of the SERAPHIN population. However, the baseline characteristics of the sub-study patients were similar to those of the overall SERAPHIN population and balanced between treatment arms, hence mitigating a potential selection bias in this non-randomized sample of the study population. Nevertheless, the results should be interpreted with caution; they are based on a small sample size, are exploratory in nature and are not adjusted for multiple comparisons. In addition, the associations found in our analysis between haemodynamic parameters and NT-proBNP and disease progression are achieved with a patient population selected for a clinical trial. Different results may be possible with diverse PAH populations and/or treatment strategies. The mechanism of action by which macitentan affects these parameters is not explained by these data and is beyond the scope of this study. This may be addressed in the on-going REPAIR (Right vEntricular remodelling in Pulmonary Arterial hyperTension) study (NCT02310672), which will evaluate the effects of macitentan on RV remodelling in PAH assessed by cardiac magnetic resonance imaging.

Conclusions

In summary, for all treatment groups in the SERAPHIN haemodynamic substudy, baseline and month 6 values of CI, RAP, and NT-proBNP, but not their changes, were associated with morbidity/mortality events. These results confirm the relevance of these parameters in predicting disease progression in patients with PAH and highlight that monitoring these parameters can provide useful prognostic information. By improving haemodynamic parameters and reducing NT-proBNP levels, macitentan increased the chance of reaching threshold values associated with lower risk of morbidity/mortality, irrespective of WHO functional class, and background PAH-specific therapy.

Supplementary material

Supplementary material is available at European Heart Journal online.

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