

The Effect of the Endothelin-1 Dual Receptor Antagonist Bosentan on Platelet Function in Patients with Pulmonary Arterial Hypertension

S. John Wort, Simon Davidson, Tamera Corte, John Park, Carl Harries and Michael Gatzoulis

National Pulmonary Hypertension Service, Royal Brompton Hospital, London, UK

Background

In-situ thrombosis is commonly seen in post-mortem specimens from patients with pulmonary arterial hypertension (PAH). Although anti-coagulation with warfarin is a standard therapy in the management of PAH, anti-platelet agents are not. This is despite mounting evidence for the involvement of activated platelets in the pathogenesis of PAH. Platelets have been shown to contain mediators proposed to be of importance in the pathogenesis of PAH, including serotonin, platelet derived growth factor (PDGF), transforming growth factor (TGF) and more recently bone morphogenetic proteins (BMPs). In addition, platelets are found to be activated in patients with PAH and it is hypothesised that they have a role in endothelial cell activation. Of the current advanced therapies for PAH, only prostacyclin is known to have an indisputable effect on platelet function. There is conflicting *in vitro* evidence for the role of endothelin-1 (ET-1) and endothelin receptor antagonists in platelet activation.

Aim

To assess the effect of bosentan, a dual endothelial receptor antagonist, on platelet function in patients with idiopathic PAH and PAH associated with other causes.

Methods

In vitro platelet aggregation:

Lumi-aggregation was used with collagen (2 µg/ml), a potent platelet stimulator, selected as the agonist. Whole blood (0.5 ml) was used to retain the larger, more active platelets, often lost when isolating platelets for other forms of platelet analysis. Experiments were performed pre- and post-bosentan (1 µM final concentration) spiking.

In vivo platelet aggregation:

Blood was taken from patients before administration of bosentan, 4 hours after administration of bosentan, 1 month after chronic dosing and 3 additional months of therapy. At all stages, plasma was separated and stored at -20°C for future ELISA analysis (PDGF, ET-1, BMP-2 and platelet glycoprotein-V).

Results

Data from healthy volunteers (Figure 2)

The impedance aggregation in healthy volunteers was 15.38 ± 5.3 ohms. Impedance aggregation was reduced to 3.75 ± 4.4 ohms after bosentan spiking. Results are expressed as mean \pm SD, $p \leq 0.001$ ($n = 10$).

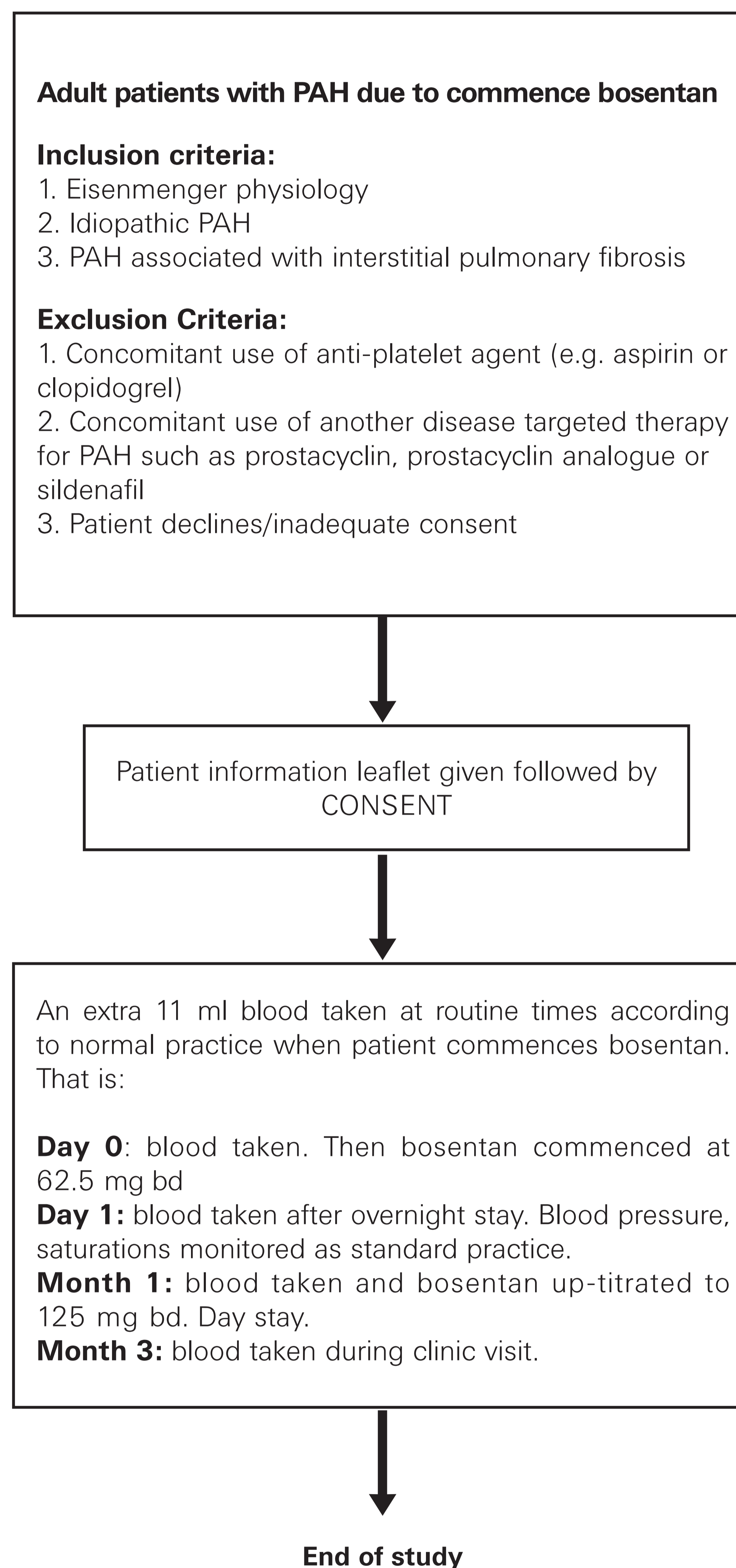


Figure 1. Clinical protocol

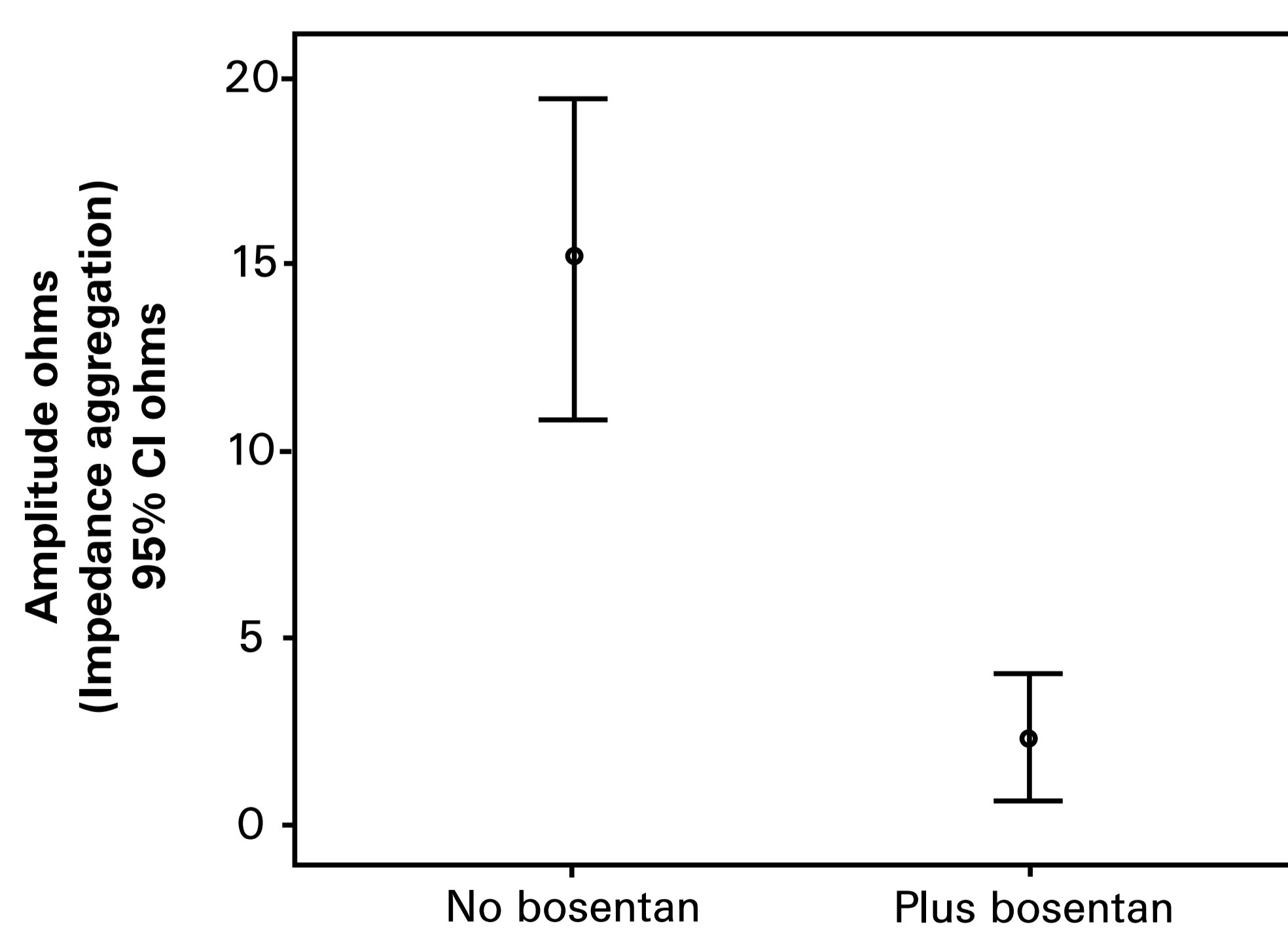


Figure 2. Healthy volunteers \pm bosentan (1 µM). Impedance aggregation (collagen 2 mg/ml), $n = 10$.

Patient data (Figure 3)

Of the 4 patients studied so far, one has idiopathic PAH and the other three Eisenmenger complex. As sample numbers are small, no statistical analysis has been performed. As in healthy volunteers, the *in vitro* spike of bosentan had a profound inhibition of aggregation. This response appeared to persist *in vivo* at day 1, although there was more variation in response. It is unclear at present whether this anti-platelet effect is maintained at one month ($n = 3$). Results are expressed as mean \pm SD.

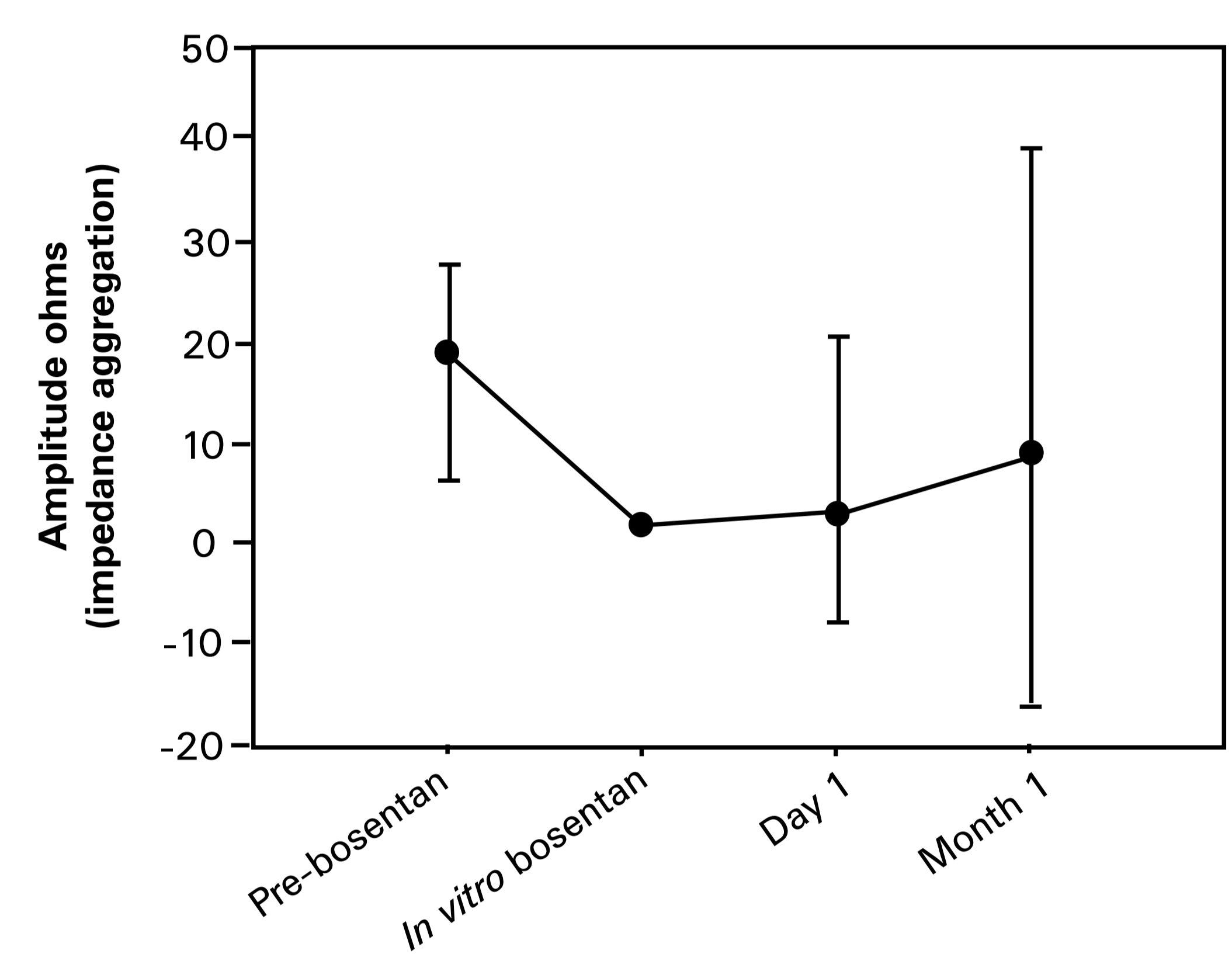


Figure 3. Patient data pre and post bosentan. Impedance aggregation (collagen 2 mg/ml), $n = 3-4$.

Conclusions

We believe that these preliminary data support the concept that bosentan has an anti-platelet aggregatory effect both *in vitro* and *in vivo*. As patient numbers are low for the *in vivo* data, we have not been able to perform statistical analysis. We are particularly interested whether patients with sub-groups of PAH will have different responses to bosentan. By the end of the study we will have looked at patients with idiopathic PAH, Eisenmenger complex and PAH associated with interstitial lung disease. We will also have performed ELISAs to assess other markers of platelet activity.