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The viewing of a 'Bloodcurdling' horror movie increases platelet reactivity: A randomized cross-over study in healthy volunteers

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ABSTRACT

Background: Epidemiological studies have suggested an increased risk of cardiovascular events (CVE) during acute stressful and/or frightful moments. A possible explanation for this could be an effect of acute stress on hemostasis. A recent study demonstrated an increase in factor VIII after watching a horror movie. Primary hemostasis, however, is thought to play a more prominent role in the etiology of CVE. The objective of this study was therefore to assess the influence of viewing a 'bloodcurdling' horror movie on platelet reactivity in healthy volunteers.

Methods: We performed a randomized cross-over study in healthy adults. Subjects were allocated to two movies in random sequence: a horror and a control movie. Blood was drawn at baseline and after 24 min of viewing time. The primary endpoint was the change in Platelet Function Analyzer® Closure Time (Δ PFA-CT) after watching the movie.

Results: In total, 20 participants, aged 18–30 years, completed the study protocol. The delta PFA-CT was statistically significantly shorter with a mean in the delta difference of −9.7 s (SEM 4.0, 95% C.I. −18.0 to −1.3) during the horror movie versus the control movie. The Light Transmission Aggregometry endpoints were in line with the PFA-CT, albeit only the highest level of Arachidonic Acid agonist demonstrated a statistically significant mean difference in the delta of aggregation of 13.15% (SEM 7.0, 95% C.I. 1.6–27.9).

Conclusion: A 'blood curdling' horror movie increases platelet reactivity. These data are supportive of a role of platelet reactivity in acute stress induced cardiovascular event risk.

1. Introduction

Abundant epidemiological data have demonstrated that – psychological - stress increases risk of morbidity and mortality from atherothrombotic cardiovascular disease [1–5]. An illustrative example is the more than doubling of cardiovascular event (CVE) risk among German men during home team soccer matches of the 2006 FIFA World Cup [6]. Although unproven, a plausible hypothesis is that events may be triggered by increases in adrenergic activity, heart rate, systemic blood pressure, and blood coagulability [7,8].

The viewing of a horror movie has been proven to induce a physiological stress response [9]. Consequently, the viewing of a horror movie can be used to simulate psychological stress in a research setting. Interestingly, a recent study demonstrated an increase in factor VIII after watching a horror movie, suggesting an effect of acute fear on the coagulation system [10]. However, primary hemostasis is thought to play a more prominent role in the etiology of CVE than secondary hemostasis [11]. Involvement of platelets is also pathophysiologically plausible because increased levels of stress hormones epinephrine and norepinephrine increase platelet activity [12]. We thus hypothesized that psychological stress, induced by viewing a horror movie, could influence platelet (re-)activity.

2. Methods

2.1. Trial design

This was a non-blinded randomized cross-over trial, comprising healthy subjects. Written informed consent was obtained from all participants. The protocol conducted in accordance with the Helsinki II Declaration and was approved by the medical ethical committee of the Amsterdam University Medical Center, Location VU University. The

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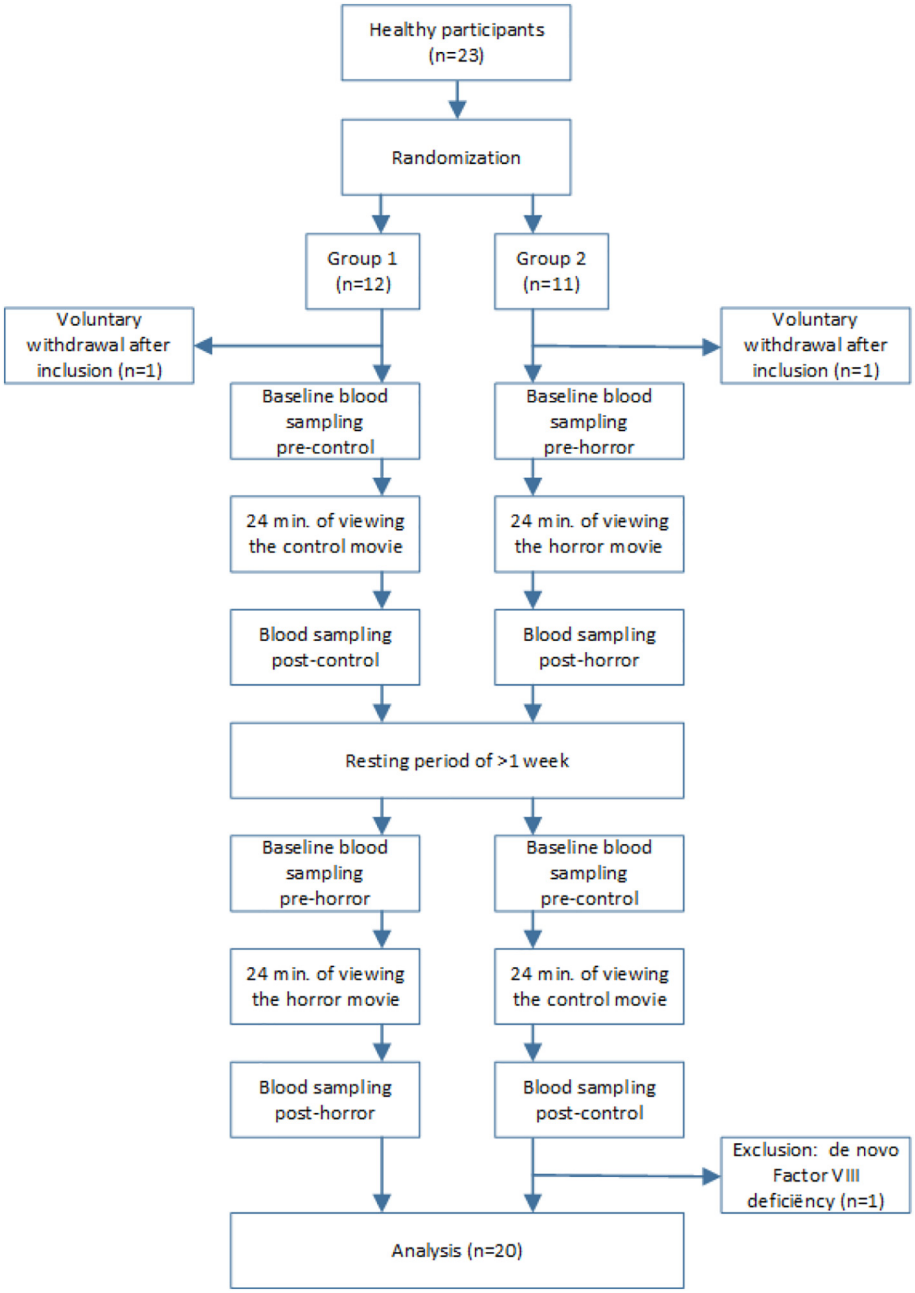

Fig. 1. Flowchart of the study protocol.

trial was registered at the Netherlands Trial Register (www.trialregister.nl - NL7824).

2.2. Participants

Participants were healthy subjects aged 18–30 years, without any prior medical history. Use of contraceptive medication with monophasic oral contraceptives or an intra-uterine device (i.e. Mirena) was permitted. Exclusion criteria were: an abnormal platelet count (< 100 or $> 400 \times 10^9$/l), recent use of antiplatelet drugs, anticoagulants or other drugs known to alter platelet function (e.g. serotonin reuptake inhibitors), hereditary hemostatic dysfunction, and pregnancy.

2.3. Study settings

A flowchart of the study protocol is shown in Fig. 1. After inclusion, randomization took place. The randomization allocation was revealed to the participants. All experiments were performed in a secluded room between 2 and 4 PM. Participants were sequentially allocated to watch 24 min (beginning of fragment at 34 min. and 56 s.) of the horror movie Grave Encounters II (Twin Engine Films; Pink Buffalo Films, Canada 2012) [13], and 24 min of the episode "Mystic Mountain" by The joy of painting with Bob Ross (2015) [14]. The timing of 24 min is based on the appearance of a bloodcurdling scene in the horror movie, the average duration of a Bob Ross episode (± 27 min), and previous research demonstrating swift effects of acute stress on blood coagulation [15]. Participants were instructed not to consume caffeinated drinks and to abstain from food and exercise 2 h before the study protocol. Moreover, female participants using a monophasic oral contraceptives were asked not apply a stop week until completing the entire protocol. Smoking and alcohol consumption were prohibited, respectively 30 min and 24 h prior to blood sampling. After having inserted the antecubital venous catheter (BD Venflon; 18GA; $1.2 * 32$ mm), participants were allowed to rest for 10 min in a semi reclined position. Blood was then

drawn from the catheter prior to watching both movies and after 24 min of viewing time of both movies. Samples were first drawn into a precursor tube, then into four sodium citrate tubes (*BD Vacutainer® 0.109 M Buff. Na_3 Citrate REF 363048)*, and finally into two EDTA tubes *(BD Vacutainer® K2E (EDTA) 7.2 mg REF 368861)*. The EDTA sample was used to measure platelet-, leucocyte-, thrombocyte count, and hemoglobin level.

2.4. End points and assessments

The primary endpoint consisted of the difference in platelet reactivity pre- and during-movie, as measured by the PFA-200. Secondary endpoints included the difference in platelet aggregation as measured by the chronolog light transmission aggregometry (LTA) and the difference in factor VIII activity levels pre- and during-movie. Laboratory personnel was not blinded to the type of exposure.

Platelet function analyzer (PFA-200): measures platelet reactivity in response to shear stress. The time needed for occlusion of an aperture cut in the collagen/adenosine diphosphate (ADP) coated membrane cartridge by the thrombocyte plug is called the closure time (CT). The CT has a maximum of 300 s [16,17].

Light transmission aggregometry (LTA): platelet rich plasma (PRP) was compared to platelet poor plasma (PPP), in which PPP served as a control. 0.105 M Sodium Citrate tubes were centrifuged at 1000*g* for 20 min, creating PRP, which was then aliquoted into one sample of 500 μl, two samples of 490 μl, and one sample of 480 μl. A magnet stir bar was added to the PRP. Remaining blood from the citrate tube was centrifuged at 1780*g* for 10 min, creating platelet poor plasma (PPP), which was then aliquoted into one sample of 500 μl. Consequently, the PPP and the four PRP test tubes were rested for circa 5 min in the chronolog aggregometer [chrono-log corporation aggregometer, series 490 dual and four channel optical aggregation systems]. The 500 μl PRP sample was placed in tracer one and was used to measure spontaneous aggregation. In tracer two and three the 490 μl PRP were placed, in which 10 μl of adenosine diphosphate (ADP), reaching a final concentration of 1 μM, and 10 μl of ethanol dissolved arachidonic acid (AA), reaching a final concentration of 0.2 μM, was added, respectively. And last, the 480 μl PRP sample was placed in tracer four, in which 20 μl of ethanol dissolved arachidonic acid was added, reaching a final concentration of 0.4 μM.

Factor VIII: One 0.105 M sodium citrate tube was centrifuged at 1780*g* for 10 min at 18 °C, followed by 5 min on 11,210 *g*. All samples were centrifuged and stored at −80 °C within 1 h after blood sampling. Plasma concentrations of coagulant factor VIII were measured by trained laboratory technicians of the hemostasis laboratory at the Amsterdam University Medical Center, Location VU University, using the STA-revolution method. According to the STA-revolution method, the plasma samples are diluted with factor VIII deficient plasma. This dilution is used for an APTT measurement, which was compared to a calibrated plasma dilution of 100%, to calculate factor VIII activity.

2.5. Stress response quantification

To estimate the level of stress, we measured heart rate, blood pressure (beat-to-beat measurement; Nexfin, BMYE Amsterdam, The Netherlands.] and skin conduction (eSense skin response app from Apple®) before and 24 min into both movies. Cortisol levels were measured from plasma stored at −80 °C before and after watching the movie. Furthermore, we conducted a questionnaire to assess subjective fear [VAS fear scale] [10].

3. Statistical analysis

3.1. Sample size

We expected that the influence of stress on the PFA-200 Closure Time would be similar to that of the variation induced by pre- and post-prandial platelet activation, previously published as 'study 1' [18]. Due to the skewed distribution of the PFA-CT, the sample size calculation was based on the Wilcoxon signed rank test. The estimated effect size was 10 s, with a power of 80%, and a two-sided alpha level of 5%. After having taking into account a possible drop-out of 10%, the total number needed to include was 22 participants (SAS version 9.2).

Depending on the distribution, data are presented as mean values ± standard deviation (SD) or median and Interquartile Range (IQR). Missing values were replaced by either mean or median, depending on the distribution. Statistical analysis was performed using paired *t*-test, or the non-parametric Wilcoxon signed-rank test when data was not normally distributed. In order to account for baseline differences, the statistical analyses were performed on the delta PFA-CT (during movie – pre-movie). Furthermore, a post-hoc sub-analysis was performed on the difference (Δhorror – Δcontrol) between female and male participants. Values of $p < 0.05$ were considered statistically significant. The software used to perform statistical analysis was SPSS version 22.0.

4. Results

4.1. Baseline characteristics

From August 2017 till June 2018, we included 23 healthy participants aged 18–30 years, of whom 21 completed the entire study protocol. The average time to complete the protocol was 11 ± 4 days. One participant had to be excluded due to a newly discovered diminished VIII activity. Overall the systolic blood pressure was 116 ± 13 mmHg, the average age was 23 ± 3, and the hemoglobin level of female participants was 7.7 ± 0.5 mmol/l, whereas that of the male participants was 9.3 ± 0.5 mmol/l. All baseline characteristics of the 20 subjects included are demonstrated in Table 1.

4.2. Stress response quantification

The Mean Arterial Pressure increased Δ5.5 mmHg (± 12.5) mmHg during the horror movie versus Δ-0.52 mmHg (± 9.4) during the control movie. The heartrate also demonstrated an increase during the horror movie (Δ7.0 ± 12.4 vs. Δ-0.9 ± 17.2) compared to the control movie. Lastly, factor VIII activity was Δ9.4% during the horror movie versus Δ4.8% during the control movie. All results are demonstrated in Table 2. Furthermore, a selection of the stress response parameters is

Table 1
Baseline characteristics.

	Total (n = 20)
General characteristics	
Age – years	23 ± 3
Female sex (n,%)	14 (70)
Systolic blood pressure in mmHg	116 ± 13
Diastolic blood pressure in mmHg	70 ± 9
BMI (kg/m^2)	22.9 ± 0.9
Alcohol intake (units/week)	5 ± 2
Caffeine intake (units/day)	3 ± 1
Current smoker (n,%)	2(10)
Use of combination oral contraceptives (COC's) (n,%[a])	
Monophasic oral contraceptives	8(60)
Intra-Uterine Device (i.e. *Mirena®)*	4(40)
Laboratory values	
Hemoglobin in mmol/l	
Female participants	7.7 ± 0.5
Male participants	9.3 ± 0.5
Leukocyte count ×10^9/L	6.9 ± 1.5
Thrombocyte count ×10^9/L	271.7 ± 56.8

[a] Percentage was calculated over female participants only; BMI, Body Mass Index; Data are presented as mean and standard deviation.

Table 2
Overview of stress parameters.

	Pre-control	During control	Pre-horror	During horror
VAS fear score	1 ± 0	1 ± 0	1 ± 0	7 ± 1
Stress induced by insertion of antecubital venous catheter[a]	4 ± 2	N/A	4 ± 2	N/A
Mean Arterial Pressure (mmHg)	84.3 ± 6.7	83.8 ± 9.0	86.7 ± 14.8	92.2 ± 15.8
Heartrate (per min)	67.9 ± 14.1	67.0 ± 21.6	69.3 ± 13.1	76.2 ± 14.6
Skin conductibility (μSiemens)	2.7 ± 2.3	3.2 ± 2.6	4.0 ± 2.5	4.8 ± 3.0
Cortisol (mmol/l)	244.0 ± 98.6	203.8 ± 96.7	254.8 ± 92.7	237.6 ± 111.5
Factor VIII activity (%)	118.8 ± 39.6	123.6 ± 39.9	131.0 ± 56.1	140.4 ± 62.7

[a] Participants were asked to rate the level of stress they endured, on a scale of 1–10, by the insertion of the indwelling venous catheter.

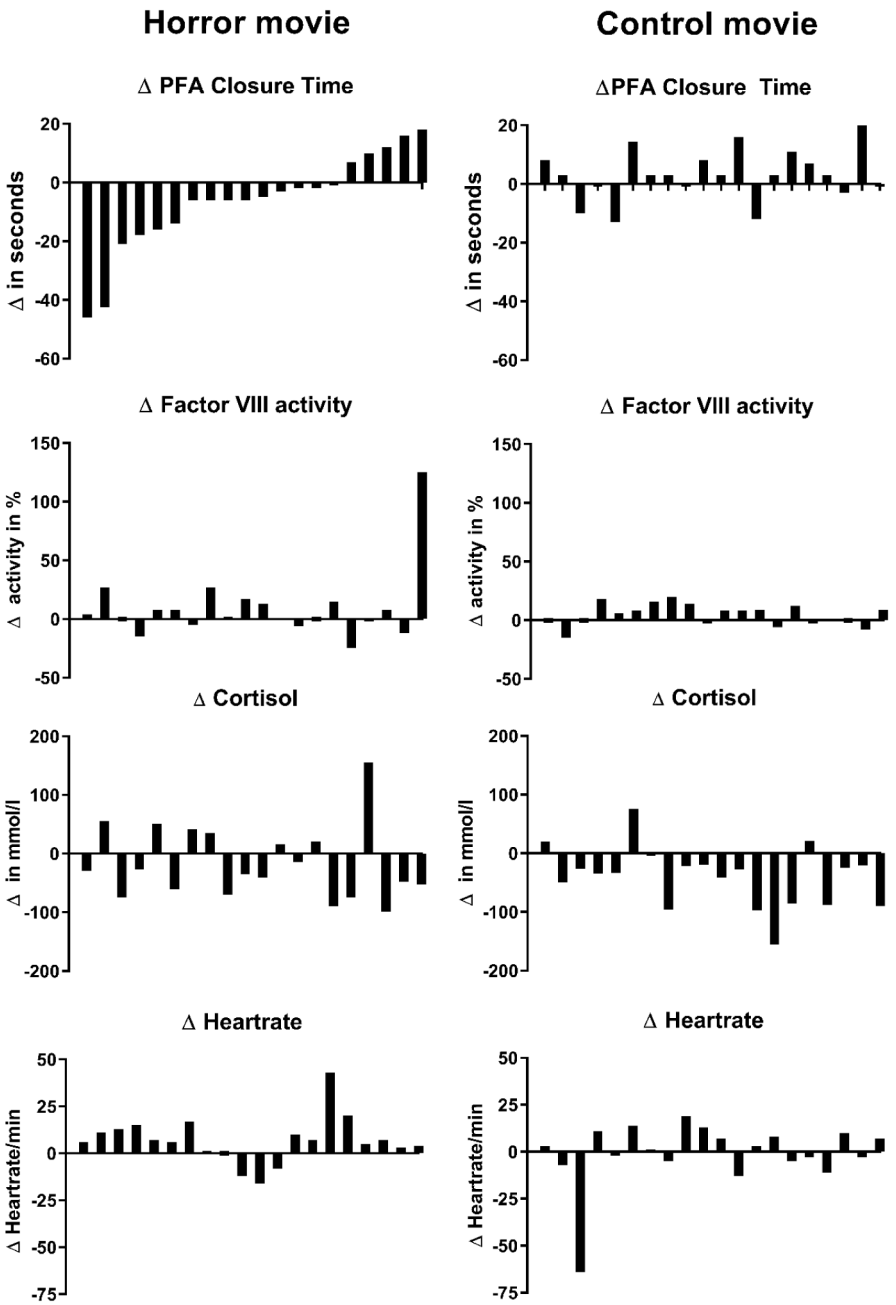

Fig. 2. Absolute mean change in the PFA Closure Time, factor VIII activity, cortisol, and heartrate, after exposure to a horror and control movie (ordered by change in the PFA Closure Time during watching a horror movie). Vertical bars represent individual participants, with order of participants identical in all graphs.

Table 3
Overview of the Chronolog LTA parameters.

	Mean difference (ΔHorror-ΔControl)	95% C.I.	*p*-Value
Chronolog LTA spontaneous aggregation	−0.65	−1.75–0.45	0.23
Chronolog LTA ADP 1.0 μM	1.63	−2.38–5.54	0.39
Chronolog LTA AA 0.2 μM	−0.90	−2.45–0.65	0.24
Chronolog LTA AA 0.4 μM	13.15	1.60–27.90	0.05

LTA, Light Transmission Aggregometry, AA, Arachidonic Acid, ADP, Adenosine Diphosphate;

also depicted in Fig. 2.

4.3. The influence of watching a horror movie on platelet reactivity

Three samples were visibly hemolytic: one pre Horror Light Transmission Aggregometry sample, and two during-horror (i.e. one Factor VIII sample and one PFA-CT). As these results were considered unreliable, these values were excluded from statistical analyses. The delta PFA-CT was statistically significantly shorter with a mean in the delta difference of −9.7 s (SEM 4.0, 95% C.I. −18.0 to −1.3) during the horror movie versus the control movie (Fig. 2). The Light Transmission Aggregometry endpoints were in line with the PFA-CT results, albeit only the highest level of Arachidonic Acid agonist demonstrated a statistically significant mean difference in the delta of max. amplitude of aggregation of 13.15% (SEM 7.0, 95% C.I. 1.6–27.9) (Table 3.),

5. Discussion

The present study suggests that acute physical stress, induced by viewing a horror movie, increases platelet reactivity as measured by the PFA-CT.. These results may, at least partly, explain the acutely increased CVE risk during acute stressful and/or frightful moments [19].

A major player inducing increased platelet reactivity during stress is the sympathetic nervous system (SNS). The SNS exerts physiological effects at times of acute stress, for example an increase in heartrate, blood pressure, and bronchodilation. Furthermore, the SNS induces adrenomedullary release of catecholamines, particularly epinephrine [20]. Catecholamines, are known to potentiate platelet activation [21]. Hence, it is likely that stress induces increased platelet activity. Interestingly, only the highest arachidonic acid agonist concentration induced a statistically significant difference in the chronolog LTA measurements. Both the PFA CADP (collagen/ADP cartridge) and the LTA AA 0.2 μM induce platelet activity by adding an overdose of agonist. It could be hypothesized that the potentiation of platelet activity is subjected to a threshold, which was not reached by the lower agonist concentrations.

Limitations of this trial include the fact that participants were aware of the nature of the trial. Hence, the participants in this trial could have been 'thrill seekers' and, perhaps, less susceptible to stress. Subsequently, the illustrated difference in platelet (re-)activity might be underestimated. Furthermore, neither in our study nor in the previously published study, the von Willebrand factor was taken into account [10]. As the von Willebrand factor is released during stress, the increased factor VIII activity could also be an epiphenomenon of the primary hemostasis rather than a direct effect on the secondary hemostasis [28–30]. However, there appears to be no correlation between the participants who demonstrated an increased platelet activity and the participants who demonstrated an increased factor VIII. And lastly, due to the non-existence of data on the relationship of increased platelet activity ex vivo and cardiovascular outcome in healthy participants, it is difficult to predict clinical meaning of the demonstrated results.

Strengths of this trial include the novelty of the research design. Although it might seem unusual, our results demonstrate that the viewing of a bloodcurdling horror movie can be easily applied to induce stress within a research setting. Furthermore, by using a cross-over design, there is a restricted influence of possible confounders. And lastly, we applied a strict adherence to the blood sampling protocol, specifically regarding the blood withdrawal to laboratory time.

6. Conclusion

A 'blood curdling' horror movie increases platelet reactivity. This finding has implications for both research and clinical practice as it elucidates the physiological influence(s) of stress and supports a role of platelet reactivity in acute stress induced cardiovascular event risk. Further work is required to establish whether this ex vivo platelet activity coincides with in vivo aggregation and if so, the viability of stress reducing interventions for the prevention of stress induced cardiovascular events.

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Statement of contribution section

1. J.J.K. van Diemen

Principal investigator, was involved with the design and conduct of the study. Furthermore, she finalized the manuscript.

2. A. van Dijk

Drs. Van Dijk was involved with the conduct of the study, as well as the first draw of the manuscript. She also approved the final draft of the manuscript.

3. C. Racca

Drs. Racca was involved with the design of the study. She also approved the final draft of the manuscript.

4. T. Knol

Drs. Knol was involved with the conduct of the study, as well as the final draft of the manuscript.

5. T.N. Bonten

Dr. Bonten was involved with the design of the study. He also approved the final draft of the manuscript.

6. M.E. Numans

Prof. dr. Numans was involved with the design of the study. He also approved the final draft of the manuscript.

7. W.W. Fuijkschot

Dr. Fuijkschot was involved with the design of the study. He also approved the final draft of the manuscript.

8. Y.M. Smulders

Prof. dr. Smulders was involved with the design of the study. He also approved the final draft of the manuscript.

9. A. Thijs

Dr. Thijs was involved with the design of the study, the statistical analyses, and the drafting of the manuscript.

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Declaration of competing interest

The authors report that they do not have any conflicts of interest to declare.

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