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Predictive parameters for spontaneous joint bleeding during emicizumab prophylaxis

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Abstract:
Emicizumab is approved for prophylaxis of patients with hemophilia A (HA). Despite its efficacy in reducing bleeding, a few patients on emicizumab still experience hemarthrosis, but no tool is yet available to identify those at higher risk of spontaneous joint bleeding. To evaluate whether laboratory measurements (global coagulation assays and emicizumab concentration) and/or arthropathy scores can distinguish patients at higher risk of spontaneous joint bleeding while on emicizumab prophylaxis. Thrombin generation assay (TGA) was assessed upon the addition of tissue factor and synthetic phospholipids. Non-activated thromboelastography (NATEM) was performed in citrated whole blood. Emicizumab concentrations were measured with a modified one-stage FVIII assay. The degree of hemophilic arthropathy was assessed with the Haemophilia Joint Health Score (HJHS) and Hemophilia Early Arthropathy Detection with Ultrasound score (HEAD-US). A Cox proportional hazards model was used to evaluate the association between variables and bleeding. The predictive power of these variables was investigated by ROC analysis. Forty HA patients with and without inhibitors on emicizumab prophylaxis were enrolled in an observational cohort study. Ten of 40 developed spontaneous joint bleeding. None of the lab parameters were able to distinguish patients at higher risk of spontaneous joint bleeding. ROC analysis showed that during emicizumab prophylaxis only the presence of synovitis and a higher HEAD-US score were associated with spontaneous joint bleeding (AUC 0.84). A greater degree of arthropathy and the presence of synovitis could help to predict the risk of spontaneous joint bleeding in HA patients on emicizumab prophylaxis.

Conflict of interest: COI declared - see note

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Clinical trial registration information (if any):

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Key point 1: No tool is yet available to identify patients at higher risk of spontaneous joint bleeding while on emicizumab prophylaxis.

Key point 2: Synovitis score and total HEAD-US score could help in predicting the spontaneous joint bleeding risk of patients on emicizumab prophylaxis.

Abstract

Emicizumab is approved for prophylaxis of patients with hemophilia A (HA). Despite its efficacy in reducing bleeding, a few patients on emicizumab still experience hemarthrosis, but no tool is yet available to identify those at higher risk of spontaneous joint bleeding. To evaluate whether laboratory measurements (global coagulation assays and emicizumab concentration) and/or arthropathy scores can distinguish patients at higher risk of spontaneous joint bleeding while on emicizumab prophylaxis. Thrombin generation assay (TGA) was assessed upon the addition of tissue factor and synthetic phospholipids. Non-activated thromboelastography (NATEM) was performed in citrated whole blood. Emicizumab concentrations were measured with a modified one-stage FVIII assay. The degree of hemophilic arthropathy was assessed with the Haemophilia Joint Health Score (HJHS) and Hemophilia Early Arthropathy Detection with Ultrasound score (HEAD-US). A Cox proportional hazards model was used to evaluate the association between variables and bleeding. The predictive power of these variables was investigated by ROC analysis. Forty HA patients with and without inhibitors on emicizumab prophylaxis were enrolled in an observational cohort study. Ten of 40 developed spontaneous joint bleeding. None of the lab parameters were able to distinguish patients at higher risk of spontaneous joint bleeding. ROC analysis showed that during emicizumab prophylaxis only the presence of synovitis and a higher HEAD-US score were associated with spontaneous joint bleeding (AUC 0.84). A greater degree of arthropathy and the presence of synovitis could help to predict the risk of spontaneous joint bleeding in HA patients on emicizumab prophylaxis.

Background

Severe hemophilia A (HA) is an X-linked bleeding disorder. Despite therapeutic advances and reduction of bleeding episodes[1], a single joint bleed is sufficient to trigger progressive joint damage [2]. Emicizumab (Hemlibra®; F. Hoffmann-La Roche), the first non-replacement therapy approved for prophylaxis of HA patients with or without inhibitors, increases thrombin generation in patients with HA[3]. The efficacy and safety of emicizumab were established by the HAVEN pivotal clinical trials [4-9], with post-traumatic events accounting for most of the bleeding. Nonetheless, spontaneous bleeding accounts for a large proportion of hemorrhagic events, from 22.2% up to 40% according to the recent literature reporting real-world data[10,11]. Several studies evaluated the thrombin generation assay (TGA)[12] and a few evaluated the Non-Activated Thromboelastometry (NATEM)[13,14] for their ability to monitor emicizumab efficacy. Nevertheless, TGA has never been standardized in the context of emicizumab prophylaxis, nor has been shown to predict the hemorrhagic risk[15]. A recent study reported a difference in NATEM clotting time (CT) between patients with and without breakthrough bleeds in a population of 63 patients on emicizumab prophylaxis, but no differences were noted between post-traumatic and spontaneous bleeding[16]. Although the usefulness of global coagulation assays during emicizumab prophylaxis is still debated and controversial, a monitoring method is lacking. Being able to predict which patients are at higher risk of developing spontaneous joint bleeding could help clinicians prevent the further progression of hemophilic arthropathy. Patients who, despite adequate prophylaxis, still experience spontaneous hemarthrosis represent an unmet need. The Hemophilia Early Arthropathy Detection with Ultrasound score (HEAD-US) proved to be superior to clinical reported hemarthrosis and to Haemophilia Joint Health Score (HJHS) in detecting early signs of joint damage[17]. The usefulness of arthropathy scores to identify patients at higher joint bleeding risk while on emicizumab has never been investigated. Given this context and knowledge gaps, a comprehensive study exploring laboratory parameters (TGA, NATEM and emicizumab plasma concentration) and clinical characteristics aimed at detecting predictive parameters of spontaneous joint bleeding was conducted to determine if a comprehensive evaluation of global coagulation assays and clinical variables might help to identify patients at a higher risk of spontaneous joint bleeding while on emicizumab prophylaxis.

Materials and methods

Study design

A prospective cohort study was performed by enrolling consecutive patients with severe HA with and without FVIII inhibitors on emicizumab prophylaxis and referring to the Angelo Bianchi Bonomi Hemophilia Center in Milan, between September 2020 and September 2022. Patients with

a body weight of less than 20 kilograms were excluded for safety reasons concerning the amount of blood required for global coagulation assays, and those with less than 6 months of follow-up were also excluded. All provided written informed consent in accordance with the Declaration of Helsinki and with institutional review board approvement.

Outcomes

Participants were instructed to contact a 24-hour phone line to report to on-call physicians any bleeding event. Joint bleeding was considered spontaneous in the absence of any external cause (trauma or intense physical exercise). Our hub Centre is equipped with a 24-hour expert medical on-call service, trained to tackle hemarthrosis. Therefore, each event was confirmed with a visit at our Centre and the data were prospectically collected.

The annualized bleeding rate (ABR) was calculated by dividing the total number of bleeds for the duration of observation period and then normalizing the results for one year.

Blood sampling

A total amount of 15 mL of blood was collected per patient into 1/10 volume of 0.105 M trisodium citrate. Patients had blood collected just before any emicizumab subcutaneous injection. In the case of a recent bleeding event, blood was drawn after at least one week from the last treatment with adjunctive FVIII or a bypassing agent.

Laboratory methods

Emicizumab concentrations were measured by using a modified one stage FVIII assay[18]. For NATEM, 300 µL of whole citrated blood were added to 20 µL of CaCl2 (100 mM) in the absence of activators, followed by recording the viscoelastic clot formation at 37 °C by using the ROTEM Delta® device (Werfen). Tracings were recorded for 9000 s and analyzed using such standardized parameters as the clotting time (CT), clot formation time (CFT), alpha angle and maximum clot firmness (MCF). The NATEM assay was performed within 30 minutes from blood sampling. For TGA, citrated blood was centrifuged at 3000 g for 20 minutes to obtain platelet poor plasma (PPP), that was aliquoted into plastic tubes, immediately frozen in liquid nitrogen and stored at - 80°C. TGA parameters were assessed according to Hemker et al.[19] using a homemade method [20], in accordance with the ISTH SSC recommendations [21]. In brief, the PPP was obtained by double centrifugation at 2500 x g for 15 minutes. The resulted PPP was activated by 1 pmol/L human recombinant tissue-factor (Recombiplastin; Werfen) and 1.0 µM/L phospholipid mixture (1:1:1 phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine; Avanti Polar Lipids,

Alabaster, AL), in the presence or absence of 2nM rabbit thrombomodulin (Haematologic Technologies, Essex, VT). In addition, a reference plasma (normal pool) was present in each run in order to reduce inter-center variability. The evaluated parameters were lag time, time to peak (TTPeak), velocity index (Vel index), the peak thrombin height (Peak T) and the endogenous thrombin potential (ETP) [22].

Arthropathy scores

The HJHS version 2.1, a validated instrument designed to assess joint health in individuals with hemophilia by evaluating nine criteria across six index joints (elbows, knees, and ankles) and gait assessment was obtained by trained physiotherapists. The HEAD-US score has been originally developed to detect early signs of joint involvement and to assess disease progression and treatment efficacy [23]. The HEAD-US score is characterized by three domains explored by joint ultrasound (synovitis, cartilage and subchondral bone) on the six main index joints (knees, elbows and ankles), with a maximum score of 8 points per joint (synovitis 0-2 points; cartilage 0-4 points; subchondral bone 0-2 points). The HEAD-US score was performed on each patient by the same expert rheumatologist by using a single machine equipped with a 5-13 MHz linear probe. The total HEAD-US score and the total synovitis sub-score taken at the beginning of the study period were considered for each patient. The total synovitis sub-score was assessed with the HEAD-US tool as a sum of total synovitis score (0 absent/minimal, 1 mild/moderate, 2 severe) in each patient. The total synovitis sub-score was analyzed as a single parameter due to its capability to identify the joint's disease activity in hemophilic patients [24]. Moreover, the relationship with the maximum synovitis score was also analyzed, in order to investigate the relationship between the bleeding and the worst synovitis score .

Adjustment covariates

Physical exercise or sport activities were classified into 4 categories based on effort intensity. A score of 0 was associated with no physical activity; 1 for low-impact activities (e.g. walking); 2 for moderate-impact activities (e.g. physiotherapy without additional use of FVIII); and 3 for high-impact activities (e.g. soccer, skiing, or Nordic walking).

Statistical analysis

Descriptive results were reported as percentages (dichotomous variables) or as medians and interquartile ranges (continuous variables). The degree of association of NATEM/TGA parameters with emicizumab plasma levels was evaluated by calculating the Spearman's rank correlation

coefficient (rho). To determine whether the differences assessed with global coagulation assays could be due to differences in emicizumab plasma concentration, the intra-individual variability of emicizumab plasma levels was calculated with the coefficient of variation (CV). To assess if laboratory parameters and clinical characteristics differed between patients with/without spontaneous joint bleeds a Cox proportional hazards model was fitted using each NATEM/TGA and clinical parameters as independent variables. The degree to which these models were able to predict the spontaneous bleeding risk was investigated by means of ROC analysis. To account for multiple measurements, the mean value of each NATEM/TGA parameter per person was used for this analysis. Statistical analyses were performed with R version 4.2.1 and IBM SPSS Statistics (version 25.0; IBM Corp., USA).

The IRB of the Promoting center Fondazione IRCCS C  Granda, Ospedale Maggiore Policlinico [via F. Sforza 28 - 20122 Milano] approved the study on the 26th April 2021. For further information: trial.istruttoria@policlinico.mi.it

Results

Study population

Forty consecutive patients on emiciżumab prophylaxis were enrolled in this observational study. Figure 1 shows that of 51 HA patients on emicizumab prophylaxis, 11 were excluded for meeting exclusion criteria (Figure 1). Median follow-up time was 77 weeks (IQR 36-97). Median patient age was 45 years (IQR 27-57), all being adults except for a 11-year-old and a 16-year-old adolescent. Ten out of 40 patients developed spontaneous joint bleeding during emicizumab prophylaxis. Regarding the group of patients without spontaneous bleeding, 6 out of 30 developed post-traumatic bleeding. In the entire cohort, the median total ABR was 0.70 (95%CI 0.51 – 0.89) and the median spontaneous joint ABR was 0.30 (0.18 - 0.43). Fifteen of 40 patients had a previous history of inhibitor development and 8 of them still had measurable FVIII inhibitory activity, despite previous attempts to inhibitor eradication. Detailed number and description of bleeding events is reported in table S1 in supplementary.

Emicizumab plasma concentration

Emicizumab was administered at the same dosage of 1.5 mg/Kg/weekly, except for one child with a dosage of 3 mg/Kg/every 2 weeks. At steady state, patients showed variable plasma emicizumab concentrations ranging from 17 to 90 ug/mL, with an inverse correlation with age. Since multiple measurements of emicizumab concentration per patient were obtained, we could calculate the

coefficient of variation (CV) to determine intra-individual variability. The within-subject CV for emicizumab plasma concentration was 10%, indicating a tendency to maintain nearly constant plasma levels at different time points.

Correlation between global coagulation assays and emicizumab plasma concentration

The correlation between the results of global assays and emicizumab plasma concentration was investigated by means of Spearman correlation coefficient. After correction for multiple measurements, MCF for NATEM and ETP and peak thrombin for TGA showed a correlation with emicizumab plasma concentration (Spearman's rho: -0.47 for MCF, 0.37 for ETP and 0.42 for peak thrombin).

Laboratory parameters and spontaneous joint bleeding

The association between the chosen outcome (spontaneous joint bleeding) and the measured laboratory variables was investigated (Table 1). According to the Cox proportional hazards model, within emicizumab plasma concentrations from 17 to 90 ug/mL, there was no association with spontaneous joint bleeding (p value 0.37, AUC 0.63). Regarding NATEM, again none of the parameters was associated with this outcome. For TGA, the variables ETP, Peak thrombin and Vel index were close to statistical significance (p-value = 0.07, 0.06 and 0.07, respectively) but none of them appeared to be a possible predictor of spontaneous joint bleeding (AUC = 0.65, 0.65 and 0.63, respectively).

Clinical characteristics, arthropathy scores and spontaneous joint bleeding

The association between the chosen outcome (spontaneous joint bleeding) and the clinical characteristics was investigated (Table 1). Although patients developing spontaneous joint bleeding during the study were older, age was not associated with the development of spontaneous hemarthrosis (p value 0.59, AUC 0.58). Also, the Body Mass Index (BMI) was not associated with the outcome. We investigated physical activities to determine their potential function as a confounding variable, but the intensity of the activities was not related to the outcome (p-value 0.28, AUC 0.65). The only predictors of spontaneous joint bleeding were the HEAD-US total score (AUC 0.78) and the total synovitis sub-score (AUC 0.79) as measured before the observation period. The same results were obtained when the maximum synovitis score was considered as variable instead of the total synovitis score (Table 1). The detailed description for each patient is reported in Table S2.

Predictive models for spontaneous joint bleeding

Based on the Cox hazards results, a model able to predict the risk of spontaneous joint bleeding was evaluated by the ROC analysis (Figure 2). Different variables expected to have relevance in determining the bleeding risk were identified from the Cox proportional hazards model. The best prediction was identified using a model considering together the total HEAD-US and the synovitis scores (AUC 0.84). Emicizumab plasma concentration between 17 to 90 ug/mL failed to add value to the latter prediction model. Moreover, no added value in the AUC was shown by adding age to the prediction model and/or physical activities.

Discussion

Emicizumab prevents bleeding in patients with severe hemophilia, converting cases with severe or moderate hemophilia into a milder phenotype, with trauma causing the onset of the majority of intercurrent bleeding episodes. Yet, a proportion of patients develop spontaneous hemorrhages, with joint bleeding accounting for most of them [25]. Because a single joint bleed is sufficient to trigger a state of chronic inflammation leading to synovial hyperplasia and angiogenesis in turn responsible for more bleeding [26], it is important to identify predictors of these bleeds in order to reduce joint damage. Our study aimed to explore the usefulness of lab investigations (emicizumab plasma concentration and global coagulation assays) and/or arthropathy scores to identify patients at higher risk for spontaneous joint bleeding. The Cox proportional hazards model failed to show a meaningful difference in emicizumab plasma concentration between patients with and without spontaneous joint bleeding, at least within the observed range of 17 to 90 ug/mL. Both the TGA and NATEM global coagulation assays failed to distinguish patients at risk for spontaneous joint bleeding. Since emicizumab plasma concentration correlated well with global coagulation assays, our cohort study suggests that neither emicizumab plasma concentration nor global coagulation assays would be beneficial for this purpose.

In a longitudinal prospective study, Barg et al. found no difference in ETP and peak thrombin between patients with and without bleeding during emicizumab [15]. However, a study involving TGA with an analysis restricted only to spontaneous joint bleeding was not performed yet. NATEM was proposed as a useful tool to monitor patients on emicizumab prophylaxis, owing to the relative absence of coagulation enhancers [13]. At variance, we were unable to demonstrate the usefulness of this assay for monitoring emicizumab prophylaxis, since there was no difference in NATEM parameters between patients with and without spontaneous hemarthrosis.

The Cox proportional hazards model revealed that only the presence and degree of synovitis as assessed with the ultrasound was strongly associated with the outcome (p-value 0.03; AUC 0.79). Also the HEAD-US score was associated with the outcome, (p-value 0.06; AUC 0.78). Indeed, the best prediction model of spontaneous joint bleeding resulted to be the total HEAD-US score and the total synovitis score considered together (AUC 0.84). Thus, in the present study, only the presence of active synovitis and a severe degree of arthropathy seemed to be predictive of spontaneous joint bleeding episodes during emicizumab prophylaxis. The synovitis score reflects the current activity of arthropathy, whereas the HEAD-US total score represents the sum of active synovitis plus past irreversible osteochondral injury [27]. Notwithstanding the documented relationship between the HJHS and the HEAD-US scores [28], the HJHS total score was not as predictive of spontaneous hemorrhage as the imaging score. Our findings support the two-hit hypothesis, with synovitis accounting for novel episodes of spontaneous bleeding [29]. Moreover, since the degree of physical activities failed to add gain to the model, the risk of spontaneous bleeding does not appear to result from sports activities or physical exertion which, on the other hand, might play a role in post-traumatic bleeds.

As older patients could be considered at higher risk of having developed severe arthropathy due to the absence of regular prophylaxis until the 1990s, we considered separately age as a risk factor, finding no statistical significance (AUC 0.58). In contrast with our findings, a previous study involving 70 patients on emicizumab prophylaxis reported that older age was associated with the development of spontaneous joint bleeding episodes. Nonetheless, the authors did not investigate the degree of arthropathy in their cases [25].

The strengths of this study were the availability of real-world data and the follow-up of a single-center cohort. One limitation of this study is the number of spontaneous joint bleeding reported in a single center cohort, however several laboratory and clinical characteristics were accurately recorded in each patient. Further research on greater number of patients with hemophilia on emicizumab prophylaxis is warranted to draw definitive conclusions around the same topics. Limitations also include the possibility of insufficient information regarding untreated bleeding, as each patient may have experienced underreported bleeding events [30]. However, our hub center tackles with a 24-hour service all events requiring FVIII or bypassing agents' administration, so that we presume that all treated bleeding episodes were documented either by call or by the patients as home treatments.

In conclusion, despite the remarkable improvement in the bleeding tendency and reduction in total ABR, one-fourth of our patients still experienced spontaneous joint bleeding during emicizumab

prophylaxis and required replacement therapies. In our study laboratory parameters (including TGA, NATEM and emicizumab plasma concentration) failed to differentiate patients with an increased risk of spontaneous joint hemorrhage. The degree of hemophilic arthropathy and the presence of synovitis resulted to be the only parameters able to detect patients at higher risk of spontaneous joint bleeding during emicizumab prophylaxis.

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Authorship Contributions

FP and SA designed the study. FP, SA, RG, EB, and VB carefully evaluated patients at different time points. SA collected the data. ES, MC, CN and CV performed the laboratory assays and critically revised the manuscript. SH performed the statistical analysis, with the contribution of SA. FP, SA and RP evaluated the results and wrote the manuscript. All authors critically revised the manuscript and approved the final manuscript for submission.

Conflicts of interest

FP received honoraria for participating at advisory boards organized by CSL Behring, Biomarin, Roche, Sanofi and Sobi.

RG received honoraria for participating as a speaker at advisory boards and seminars organized by Pfizer, Roche, Novo Nordisk, Takeda, outside the present work.

The other authors stated that they have no interests which might be perceived as posing a conflict or bias.

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Figure 1: Study flowchart.

Figure 2: ROC analysis. HEAD-US total score and total synovitis score in the prediction model (AUC 0.84).

Table 1: Distribution of parameters between spontaneous joint bleeders (SJB) and non-spontaneous bleeders (NSB). Hazard ratio, P-value and Area Under the Curve (AUC) extracted from a Cox proportional hazards model are shown. IQR, interquartile range. 95%CI, 95% Confidence Interval. Peak T, Peak Thrombin. TTPeak, Time to Peak. Vel I, Velocity Index.

Variable	SJB median (IQR)	NSB median (IQR)	Hazard ratio (95%CI)	p-value	AUC
N° of patients	10	30			
Age	55 (41 - 61)	41 (28 - 52)	1.0 (0.97-1.06)	0.59	0.58
BMI	23.6 (20.1 - 28.2)	23.7 (21.4 - 24.7)	0.96 (0.84-1.1)	0.51	0.52
Activity intensity	1 (0 - 1)	1 (0 - 2)	0.7 (0.4-1.3)	0.28	0.65
HJHS	25 (14 - 35)	13 (3 - 16)	1.0 (0.98-1.1)	0.19	0.66
HEAD-US	24 (20 - 25)	7 (1 - 11)	1.08 (1.0-1.2)	0.06	0.78
Synovitis total score	1 (1 - 2)	1 (0 - 1)	3.05 (1.15-8.12)	0.03	0.79
Synovitis max score	1 (1 - 2)	1 (0 - 1)	4.6, (1.0 - 7.1)	0.04	0.77
Emicizumab	40.9 (33.3-55.2)	51.0 (42.2 - 63.1)	0.98 (0.95-1.0)	0.37	0.63
NATEM					
CT (s)	865.0 (755.0-1006.5)	832.4 (722.1-899.8)	1.0 (0.99-1.0)	0.26	0.62
CFT (s)	176.0 (145.5-237.6)	187.7 (164.4-222.7)	1.0 (0.99-1.0)	0.49	0.55
MCF (mm)	60.5 (56 - 63)	58.3 (55.5 – 60.6)	1.0 (0.92-1.1)	0.73	0.45
MCF.t (s)	1684.5 (1612-1701)	1712.8 (1485-1855)	1.0 (0.99-1.0)	0.94	0.41
Alpha (°)	57.0 (51.8-63.0)	57.8 (54.5-61.0)	0.96 (0.89-1.0)	0.42	0.58
TGA					
Lag time (min)	8.3 (7.8-12.1)	8.1 (7.4-8.8)	1.06 (0.8-1.4)	0.71	0.41
ETP (nM · min)	785.1 (632.7-997.0)	1053.0 (993.6-1282.1)	0.99 (0.99-1.0)	0.07	0.65
Peak T (nM)	51.1 (38.4-63.5)	77.3 (64.5-94.2)	0.96 (0.94-1.0)	0.06	0.65
TTPeak (min)	20.8 (20.2-24.2)	18.6 (17.8-19.5)	1.06 (0.89-1.3)	0.52	0.57
Vel I (nM/min)	4.8 (3.0-5.2)	7.7 (6.5-9.4)	0.75 (0.5-1.0)	0.07	0.63

Figure 1

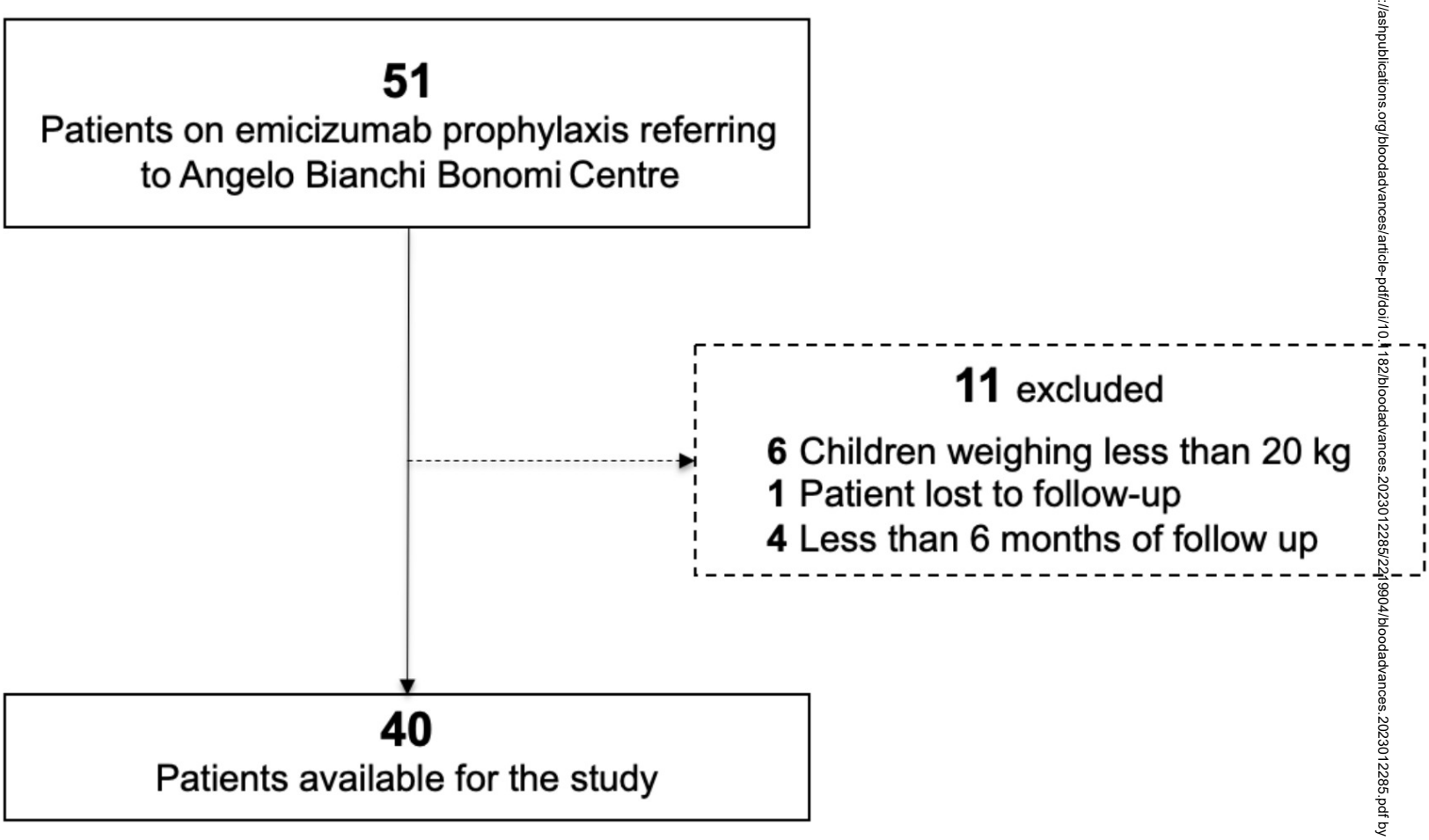

Figure 2