Propranolol for treatment of familial cerebral cavernous malformations (Treat_CCM): a phase 2, randomised, open-label, blinded endpoint pilot trial

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SUMMARY

Background Observations in people with cerebral cavernous malformations, and in preclinical models of this disorder, suggest that the beta-blocker propranolol might reduce the risk of intracerebral haemorrhage. We did an exploratory trial to test whether chronic treatment with propranolol would reduce the incidence of clinical events (symptomatic intra-cerebral haemorrhage or focal neurological deficit) in people with familial cerebral cavernous malformation.

Methods Treat_CCM is a phase 2, randomised, open-label, blinded endpoint pilot trial conducted at six Italian hospitals. People with symptomatic familial cerebral cavernous malformation aged 18 years or older were included. Participants were randomised (2:1) to either oral propranolol (20–320 mg daily) and standard care (intervention group), or to standard care alone (control group), for 24 months. Participants, caregivers, and investigators were aware of the random assignment. Investigators did clinical assessments and 3 T brain MRI at baseline and at 12 and 24 months. The primary outcome was new occurrence of symptomatic intra-cerebral haemorrhage or focal neurological deficit attributable to cerebral cavernous malformation. Outcome assessors were unaware of the random assignment. The primary analysis was done in the intention-to-treat population. Because of the pilot study design, we chose a one-sided 80% CI, which could either exclude a clinically meaningful effect or show a promising signal of activity. Treat_CCM is registered with EudraCT (2017-003595-30) and ClinicalTrials.gov (NCT03589014); recruitment is closed.

Findings Between April 11, 2018, and Dec 5, 2019, 83 participants were enrolled to the study, of whom 57 were assigned to the intervention group and 26 to the control group. Mean age of participants was 46 years (SD 15), and 48 (58%) were women. The incidence of symptomatic intra-cerebral haemorrhage or focal neurological deficit in the intervention group was 1·7 (95% CI 1·4–2·0) cases per 100 person-years (two of 57), and in controls it was 3·9 (95% CI 3·1–4·7) cases per 100 person-years (two of 26). The univariable hazard ratio for symptomatic intra-cerebral haemorrhage or focal neurological deficit (0·43, 80% CI 0·18–0·98) is a promising signal, according to predefined criteria. The incidence of hospitalisation did not differ between groups (8·2 [95% CI 7·5–8·9] in the intervention group versus 8·2 [95% CI 7·1-9·3] in the control group cases per 100 person-years). One participant who was assigned to the control group died of sepsis. Three participants assigned to the intervention group discontinued propranolol due to side effects: two reported hypotension and one weakness.

Interpretation Propranolol was safe and well tolerated in participants. It might be beneficial for reducing the incidence of clinical events in people with symptomatic familial cerebral cavernous malformations, although our trial was not designed to be adequately powered to investigate efficacy. A definitive phase 3 trial of propranolol in people with symptomatic familial CCM appears justified.

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Research in context

Evidence before this study

We searched PubMed from database inception to May 30, 2022, with the terms "cerebral cavernous malformation" and "propranolol", without language restriction. We found seven case reports (five in infants or children) reporting benefits of propranolol for cerebral cavernous malformation in humans. We found four cohort studies investigating outcomes for patients with sporadic or familial cerebral cavernous malformation associated with beta-blocker use: three retrospective cohort studies did not find any associations between beta-blocker use and outcomes, although one prospective, population-based study found an association between beta-blocker use and a lower risk of intra-cerebral haemorrhage or new persistent/progressive focal neurological deficit adjusted for known predictors of these outcomes (adjusted hazard ratio, 0·09 [95% CI, 0·01-0·66]; p=0.018). We searched ClinicalTrials.gov on May 30, 2022 for "cerebral cavernous malformation" and "propranolol" and found, besides Treat_CCM, two randomised clinical trials for patients with surgically inaccessible cerebral cavernous malformation (NCT03523650) and another for people undergoing surgery for cerebral cavernous malformation (NCT03474614); both clinical trials were of unknown status and are not published.

Added value of this study

The Treat_CCM trial is the first Phase 1/2 randomised, controlled trial assessing the safety and efficacy of propranolol for cerebral cavernous malformation. Existing data on propranolol in CCM come from retrospective analyses of cohorts and anecdotal case reports. The prospective design of this multicentre study along with randomization of included subjects allows for reduction of potential bias and increases generalisability of the findings.

Implications of all the available evidence

We found that propranolol was safe and well tolerated for the treatment of familial cerebral cavernous malformation. The effects on clinical outcomes and new occurrence of cerebral cavernous malformation on MRI were not significant, but their direction and magnitude suggest that propranolol might be beneficial. Altogether, pre-clinical work in animal models, case reports and observational studies in humans, and the results of Treat_CCM justify a definitive main phase clinical trial of propranolol for preventing intra-cerebral haemorrhage and focal neurological deficit from cerebral cavernous malformation.

INTRODUCTION

Cerebral cavernous malformations (CCMs) are vascular malformations characterized by clusters of enlarged leaky capillaries mainly affecting the central nervous system. They represent the second most common type of vascular malformation, with a reported prevalence ranging from 0.1% to 0.8% in the general population.[1] Most CCM are solitary and sporadic, of unknown cause, whereas multiple CCM are usually familial CCM (fCCM). fCCM is a rare genetic disease with an estimated population prevalence of 1/5,000 to 1/10,000 (Orphanet) arising from autosomal dominant inheritance of loss-of-function mutations in one of three genes: *KRIT1* (*CCM1*), *CCM2* (Malcavernin) and *PDCD10* (*CCM3*).[2]

CCMs can cause intra-cerebral haemorrhage (ICH), non-haemorrhagic focal neurological deficit (FND), or epileptic seizure(s) and may cause severe disability. The five-year risk of intra-cerebral haemorrhage ranges from 3.8% to 30.8%.[3] Without effective medical treatment to prevent intra-cerebral haemorrhage from CCM, the main therapeutic option is neurosurgical excision of solitary CCM, which is offered to the minority of patients with symptomatic CCM located in safely accessible locations,[4] or stereotactic radiosurgery which is reserved for such CCM unsuitable for surgery. Patients with fCCM remain at risk from other CCM despite treatment of the symptomatic lesion. Drug treatment of fCCM could reduce the burden of disease and avoid invasive treatment of the symptomatic lesion.

Other than a pilot phase clinical trial of simvastatin in 12 participants with fCCM[5], and an ongoing phase 1-2 clinical trial of atorvastatin in 80 participants with CCM (NCT02603328), no clinical trials have assessed potential disease-modifying treatments for fCCM.[4] Propranolol, a non-selective β-adrenergic receptor-blocking agent, is effective for soft-tissue infantile haemangioma,[6–11] a condition similar to CCM. Animal models have suggested that propranolol could also stabilise CCM via effects on inflammation, angiogenesis and the pericyte-endothelial cell interaction.[12,13] In humans with CCM, several case reports have reported CCM regression or stabilisation with propranolol.[14–16] One non-randomised cohort study of humans with sporadic CCM and fCCM have found an association between any beta-blocker and a lower risk of intra-cerebral haemorrhage from CCM after adjusting for known predictors of intra-cerebral haemorrhage,[17] although other cohort studies had not shown this.[18–20]

Therefore, we initiated a phase 2 pilot clinical trial comparing the effect of propranolol with standard care versus standard care alone for subjects with both fCCM and sporadic CCM on safety, clinical efficacy, and MRI appearances.

METHODS

Study design and participants

Treat_CCM is a multicentre, randomised, open-label, blinded endpoint, phase 2 pilot trial at six national reference centres for rare diseases across Italy (see list at the end of the report). The study protocol has been published.[21]

Eligible patients with CCM were aged ≥18 years and had a history of clinical symptoms of intra-cerebral haemorrhage, seizures, stroke, permanent or transient focal neurological deficit , intellectual disability, or any other neurological symptoms supposedly related to CCM. Patients unable to give their informed consent and to adhere to the study procedures were not considered for inclusion. Exclusion criteria included: implanted pacemaker or any other condition contraindicating the magnetic resonance imaging (MRI); bradycardia (<50 bpm); 2nd or 3rd degree atrioventricular block; symptomatic hypotension; unstable diabetes; severe asthma; renal and/or liver failure; current use of verapamil or diltiazem; brain surgery within 6 months; known hypersensitivity to study drug (propranolol or any of the ingredients); pregnant or lactating women, or women of childbearing potential not protected from pregnancy by an accepted method of contraception; participation in another clinical trial.

The study was approved by local research ethics committees and all participants provided written informed consent at the first visit before any study procedures or assessments. The trial was conducted according to all the stipulations of the protocol, intra-cerebral haemorrhage E6 Guideline for Good Clinical Practice (GCP) requirements, and the applicable regulatory requirements.

Randomisation and masking

After obtaining written consent and verifying inclusion and exclusion criteria, eligible patients were randomly assigned in a 2:1 ratio to receive either propranolol with standard care or standard care alone. A randomized blocks list was generated through a C language program to define the randomization list. A block of dimension 6 was used, and blocks were assigned in equal number to the participating centres to implement a stratification by centre. The web-based randomization system gave the code number and study treatment to the investigators, after correct introduction of data documenting the presence of eligibility criteria and the absence of any exclusion criterion. The trial was open-label for patients, carers and study investigators. The investigators involved in event adjudication and MRI analysis were masked to treatment assignment, adhering to a PROBE (prospective, randomised, open-label, blinded endpoint) design. Blinding of all event documentation and of MRI recordings was performed by trained personnel at the Study Secretariat at Mario Negri.

Procedures

Participants had blood analyses to check for normal liver and kidney function,

electrolyte and blood glucose at baseline, 12 and 24 months. We performed CCM1 (KRIT1), CCM2 (MGC4607) and CCM3 (PDCD10) mutation analysis on all participants during follow-up, for those who did not have it done before randomisation.

Propranolol was administere with standard care orally with a recommended initial dose of 40 mg twice daily, to be up-titrated to 80 mg twice daily. However, we amended the study protocol to allow for doses as low as 10 mg twice daily and up to 160 mg twice daily, for a total dose of 20 mg to 320 mg daily, according to tolerability. Clinical monitors checked participants' adherence during monitoring throughout the study by comparing the amount of propranolol prescribed versus the amount returned used or unused. The analysis of propranolol in plasma was performed by High Performance Liquid Chromatography – Mass Spectrometry (details in **Supplementary materials**).

Except for brain surgery, which was a criterion for exclusion, any drug deemed necessary for patients was allowed as part of standard care, including NSAIDs, anticonvulsants and antithrombotic agents.

We performed follow-up clinical visits at weeks 2 and 4 to adjust the dosage of propranolol, then every 6 months until study end, month 24. The clinical follow-up visits at baseline, 12 and 24 months included a clinical examination, blood pressure and heart rate measurement, a full neurological examination, modified Rankin Scale (mRS), electrocardiogram (ECG) and blood sampling. We performed the 6- and 18-month follow-up visits either in person or by telephone to check for drug tolerability and occurrence of adverse events. Blood chemistry analyses, including vitamin D and high-sensitivity C-reactive protein (hsCRP), were performed using conventional methods in a Clinical Chemistry laboratory (Desio Hospital, Italy).

Participants underwent 3T cerebral MRI according to a dedicated protocol in five site-specific MRI scanners at baseline, 12 and 24 months. The MRI protocol included: sagittal 3D T1-weighted Turbo Field Echo (TFE), sagittal 3D T2-weighted Turbo Spin Echo (TSE), sagittal 3D fluid attenuated inversion recovery (FLAIR), axial diffusion weighted imaging (DWI), axial susceptibility weighted imaging (SWI) and axial T2-weighted Gradient Echo (GRE). We did multiplanar reconstruction (MPR) for 3D sequences.

Outcomes

The primary outcome was the occurrence of new clinically symptomatic intra-cerebral haemorrhage or focal neurological deficit attributable to CCM over 24 months.[22] Pre-specified secondary outcomes were microvascular haemorrhages (assessed by quantitative susceptibility mapping), patient-reported clinical outcomes other than intra-cerebral haemorrhage and focal neurological deficit (global cognitive function, global disability assessed by modified Rankin Scale, health related quality of life [assessed with SF-36]), seizures, and vascular lesion characteristics, as assessed by MRI (including number, diameter, and length of CCM, location [cerebellum,

brainstem, basal ganglia, and hemispheric white matter], volume of the largest CCM, appearance of de novo CCM, and signs of new bleeding at 12 and 24 months. MRI signal of CCMs was reported according to the Zabramski classification.[23] The analysis of some secondary outcomes—i.e., microvascular haemorrhages and patient-reported outcomes—will be the subject of separate publications.

The severity of adverse events was evaluated using the Common Terminology Criteria for Adverse Events. The investigators also assessed the causal relationship between adverse events and the use of propranolol or the study procedures.

Statistical analysis

We estimated a 10.1% 2-year risk of the primary outcome in fCCM patients with standard care.[3] Assuming a 50% reduction of clinical events with propranolol, at least 834 patients (556 propranolol : 278 control) would have been needed to achieve a study power of 80% at a significance level of one-tail alpha=0.05[24], an unrealistic scenario. Treat_CCM, a pilot study, adopted a confidence interval approach.[25] Following a pilot study design, a one-sided 80% CI was chosen, because we were interested in proceeding toward a main trial only if some evidence of effectiveness could be seen. If propranolol is found to be safe with no excess of adverse events, 60 patients randomised (2:1) to either propranolol or control a clinically meaningful effect, defined as ≥50% reduction of the 2-year risk of the primary outcome, can be excluded by the defined 80% upper confidence interval. Otherwise, the study will provide a promising signal of activity. Thus, if the constructed 80% confidence interval excludes 1, the results are considered as promising.

Baseline characteristics are presented by treatment groups, as means ± SD, median [Q1-Q3] or N (%), as appropriate. We performed the main analyses of the safety and primary efficacy outcomes according to an intention-to-treat approach, including all 83 participants who were randomised. We conducted secondary per-protocol analyses, restricted to participants with fCCM with proven genetic mutation and excluding non-adherent participants. Serious adverse events, defined as hospitalisation for any reason, as well as primary endpoint events (i.e. intra-cerebral haemorrhage or focal neurological deficit), are reported per treatment group as incident rate per 100 person-years (including Poisson exact 95% CI). Univariable unadjusted Cox regression analysis was performed with a 80%CI for the primary endpoint, including all available follow-up in order to assess whether the results of the trial can be considered encouraging. For the radiological secondary outcomes, we excluded participants who underwent neurosurgical excision of CCM, those without genetically confirmed fCCM and those without baseline MRI. Analyses were performed using IBM SPSS Statistics for Windows, version 27 (IBM Corp., Armonk, N.Y., USA).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or report drafting.

RESULTS

Between Apr 11, 2018 and Dec 5, 2019, investigators assessed 95 patients, of whom four met exclusion criteria and eight did not provide consent, leaving 83 participants who were deemed eligible and randomly assigned, 57 to propranolol with standard care and 26 to standard care alone (**Figure 1**). 12 participants (9 randomized to propranolol and 3 to standard care) were deemed ineligible after review of baseline MRI due to: normal MRI (n=1), leukoencephalopathy with microbleeds (n=1), radiation-induced CCM (n=1), and sporadic CCM without genetic mutation (n=9).

Baseline characteristics were generally well balanced between groups (**Table 1**). The mean age of participants was 45·8 years, 48 (57·8%) were female, and all patients were Caucasian. The most common prior symptom related to CC\M was recurrent headache (71·1%), followed by intra-cerebral haemorrhage (57·8%), focal neurological deficit (48·2%) and epileptic seizures (37·3%). Participants assigned to propranolol with standard care more frequently had a history of focal neurological deficit compared to standard care (54·4% vs 34·6%). 71 (85·5%) participants had fCCM with a known genetic mutation (48 assigned to propranolol with standard care versus 23 assigned to standard care). Thirteen patients were prescribed a statin: 4 in controls and 9 in propranolol. In addition, 10 patients were on vitamin D supplementation, 2 in controls and 8 in propranolol (table 1).

79 (95·2%) of participants adhered to the treatment assigned by randomisation; three (5·3%) participants discontinued propranolol 10 weeks, 6 months and 18 months after being assigned to it, and one (3·8%) participant started propranolol 20 mg twice daily six months after being assigned to standard care on his/her own initiative. Propranolol was not detectable in blood samples at two years in nine participants assigned to propranolol with standard care (five participants from one site had sporadic CCM) and in all but two participants assigned to standard care. Daily doses of propranolol ranged from 20 mg to 160 mg during two-year follow-up; a dose of propranolol of at least 40 mg per day was prescribed for 40 (70·2%) of 57 participants and the median prescribed daily dose over 24 months was 50 mg. Low daily doses ranged from 20 to 40 mg, appropriate daily doses ranged from 40 to 160mg. Propranolol concentrations in plasma were measured at participants' 2-year visit, when median concentration was 27·5 ng/mL (IQR 9·8-61·0) in participants assigned to propranolol. Participants prescribed a daily dose of propranolol ≤50 mg (n=33) had a median circulating propranolol concentration of 15·8 ng/mL (7·3-35·8) while participants prescribed ≥60 mg (n=24) had a median concentration of 54·9 ng/mL (21·8-89·0).

Only one (1·2%) participant, assigned to standard care, was lost to follow-up immediately after performing baseline visit. The median duration of follow-up was 764 (IQR 736-808) days, little over two years, due to COVID-19 pandemic.

Among 83 participants in the intention-to-treat population, the primary clinical

outcome of new symptomatic intra-cerebral haemorrhage or focal neurological deficit attributable to CCM occurred in two (3·5%) of 57 participants assigned to propranolol with standard care (incidence 1·7 [95% CI 1·4-2·0] per 100 person-years) versus two (7·7%) of 26 participants assigned to standard care alone (incidence 3·9 [95% CI 3·1-4·7]; hazard ratio 0·425 [80% CI 0.183-0.984]; **Figure 2A**). This confidence interval excludes equivalence and therefore the study provides a promising signal of activity as determined in the protocol.

The two intra-cerebral haemorrhages caused transient neurological deficit in one participant and permanent neurological deficit in the other; the two focal neurological deficits were not disabling and in one of them symptoms were transient (**Supplementary table 1**). All four participants who experienced at least one primary outcome had fCCM with a confirmed genetic mutation (**Supplementary table 1**). The secondary clinical outcome of epileptic seizure during follow-up affected two (3·5%) participants assigned to propranolol with standard care (incidence 1·7 [95%CI 1·3-2·0]) and one (3·8%) participant assigned to standard care (incidence 1·9 [95%CI 1·4-2·5]).

The frequency of hospitalization for any reason was similar in both groups: there were 11 hospitalisations in nine (15·8%) participants assigned to propranolol with standard care and six hospitalisations in four (15·4%) participants assigned to standard care (incidence 8·2 [95%CI 7·5-8·9] versus 8·2 [95%CI 7·1-9·3]; **Figure 2B**). We adjudicated nine hospitalisations as unrelated to CCM and none was deemed related to propranolol (**Supplementary table 2**). One participant assigned to standard care died, due to sepsis. No other serious adverse events were reported.

Propranolol was well tolerated. 11 (19·3%) of 57 participants assigned to propranolol with standard care experienced transient symptomatic episodes of hypotension (i.e. systolic BP <90mmHg and diastolic BP <60 mmHg) or bradycardia (i.e. heart rate <50 beats per minute). 35 participants reported 78 transient side effects, the most common being fatigue (34 episodes in 19 participants), hypotension (19 episodes in 16 participants) and bradycardia (17 episodes in 13 participants). No abnormalities attributable to propranolol were found on ECG during the trial. Systolic and diastolic BP fell by a mean of 6·1 mmHg and 4·9 mmHg respectively and heart rate fell by a mean of 7·8 beats per minute after one year in the group assigned to propranolol (**Supplementary Table 4**).

Of 71 participants with fCCM and evidence of a genetic mutation (**Table 1**), 48 were assigned to propranolol with standard care and 23 to standard care alone. Baseline characteristics were similar between groups (**Supplementary table 5**). The incidence of the primary clinical outcome of new symptomatic intra-cerebral haemorrhage or focal neurological deficit attributable to CCM was 2·0 (95% CI 1·6-2·4) in participants assigned to propranolol with standard care versus 4·3 (95% CI 3·4-5·1) after standard care alone. The incidence of hospitalisation was 7·5 (95% CI 6·7-8·3) in participants assigned to propranolol with standard care versus 9·0 (95% CI 7·8-10·2) after standard care alone.

The per-protocol analysis (excluding patients not adhering to study treatment and those with sporadic CCM) included 68 patients, of whom 46 was assigned propranolol and 22 standard care (**figure 1**). The primary clinical outcome of new symptomatic intra-cerebral haemorrhage or focal neurological deficit attributable to CCM occurred in two (4·3%) of 46 participants assigned to propranolol (incidence 2·1 [95% CI 1·7-2·5] per 100 person-years) versus two (9·0%) of 22 participants assigned to standard care (incidence 4·5 [95% CI 3·6-5·4]; hazard ratio 0·46 [80% CI 0.128-1.662]). The incidence of hospitalisation was 7·9 [95% CI 7·1-8·7] in patients randomised to propranolol with standard care versus 6·7 [95% CI 5·7-7·8] per 100 person-years for standard care alone.

Brain MRI at baseline, 1 year and 2-year follow-up was available for 68 participants with fCCM. After the exclusion of one participant who underwent neurosurgical excision of CCM before completing the 2-year follow-up, MRI appearances were rated and analysed for 67 participants (**Table 2**). The median numbers of supratentorial and infratentorial CCM were well balanced between groups at baseline. The median total number of CCM increased over two years of follow-up in both groups overall and in supratentorial and infratentorial locations. During two-year follow-up, the median number of *de novo* CCM was 4 (IQR 2-9) after propranolol with standard care versus 5 (IQR 1-11) with standard care alone (**Supplementary table 6**). The formation of 5 or more new CCM was found in 5 participants (71·4%) taking low dose propranolol and 16 participants (40·0%) taking an appropriate dose, see Supplementary table 6, while the incidence in the 20 participants assigned standard care was 11 (55·0%).

DISCUSSION

To the best of our knowledge, Treat_CCM is the first completed pilot phase randomised controlled trial of propranolol for fCCM and the largest randomised controlled trial for any form of CCM. We found that propranolol seems safe, well tolerated, and has a promising effect for preventing intra-cerebral haemorrhage and focal neurological deficit for fCCM, although this trial was not designed to be adequately powered to investigate efficacy. The magnitudes and directions of the estimated effects on efficacy and safety clinical outcomes were consistent in the intention-to-treat analysis and the per-protocol analysis restricted to fCCM with a genetic mutation. The observed event rate for the primary outcome was similar to the estimate that informed the design of the trial.[3]

The MRI sub-study suggested that propranolol might not affect pre-existing CCM size but might reduce the number of new CCM over two years, consistent with one possible mechanism of action of propranolol observed in pre-clinical studies.[1312] However, the mechanism of action of propranolol for CCM remains poorly understood. This molecule has a pleiotropic effect on vascular permeability and angiogenesis and was found to rescue the function of the endothelium and to reduce *de novo* CCM formation in preclinical models of CCM,[12,13] although propranolol did not significantly reduce intra-cerebral haemorrhage in murine models.[12] Different mechanisms have been proposed such as beta-1 adrenergic receptor blockade,[12] or a morphological / functional improvement of pericyte-endothelial cell association, which is altered in CCM.[13]

This study has strengths. Our methods reduced selection bias by random sequence generation using computerised blocks and allocation concealment. We reduced detection bias by masking outcome assessment to assigned treatment, although we could not reduce performance bias by blinding participants and personnel to the intervention and comparator (although many of our outcomes were objective and most may not have been affected by performance bias). Completeness of follow-up was excellent, thereby minimising attrition bias. We have reported all of the outcomes pre-specified in the protocol, in order to avoid selective outcome reporting, and analysed the data according to our pre-specified statistical approach.

This study has some limitations. First, Treat_CCM recruited more than its target sample size. The Steering Committee decided to include as many patients as possible within the 18-month inclusion period, given the expected low incidence of endpoint clinical events. In addition, this allowed us to compensate for the unplanned recruitment of patients with sporadic CCM due mostly to a single centre where study protocol was incorrectly applied. 83 participants were recruited and randomly assigned, although only 71 (85·5%) had fCCM confirmed by genetic testing. Nine~~Ten~~ patients had sporadic CCM and three (4·2%) participants had normal brain MRI. Nine

of 12 participants without fCCM were recruited at one site, and propranolol was found undetectable in the plasma of the participants at this site, leaving concern about trial integrity at this site. However, because of the over-recruitment, we could correct this unexpected recruitment of patients with sporadic CCM. Although headache is a relatively weak criterion for inclusion as CCM, patients later identified without fCCM mutation were equally distributed over the different qualifying clinical signs and symptoms (i.e. epilepsy, focal neurological deficit , intra-cerebral haemorrhage).

A second limitation is that half of the participants assigned to the intervention group did not reach 80 mg/day propranolol, a dose that is conventionally considered as pharmacologically effective. Moreover, we did not implement a standardised dosing approach for propranolol. The minimum therapeutic dose of propranolol for fCCM in humans, as recently reported in animal models,[26] is unknown. In this exploratory pilot phase trial, a variable dosage of propranolol was allowed. Many patients did not tolerate (according to the investigator's judgment) 40 mg twice a day, so we introduced a protocol amendment (on Nov 30, 2018) to allow for lower doses to be given, thereby avoiding loss of patients. Also, propranolol has a bioavailability >90% but 30 to 70% is metabolised upon first passage though the liver with large inter-individual variability.[27] In a pharmacokinetics and pharmacodynamics study in healthy volunteers, average trough plasma concentrations of 20 to 30 ng/mL at steady-state were associated with a significant decrease in heart rate.[28] The median concentration of 27·5 ng/mL found in the participants in Treat_CCM suggests that it was within a pharmacologically effective range. This finding is reassuring assuming that beta-blockade is the mechanism of action in CCM, though this is not consistently proven.[12,13] The trend in reduction of new CCM lesion, although non-statistically significant, suggests a dose-response effect of propranolol in reducing incident CCM. In any future phase 3 trial, the minimum dose of 40 mg twice a day should be adhered to, with online monitoring of prescribed dose regimens.

A third potential limitation was our choice of a one-sided 80% CI in the sample size calculation. We took this approach because we were conducting a pilot trial, and it allowed us to identify a sample size that would give us reasonable confidence in the result while not requiring too large a sample size (which increases the cost, time taken to conduct the pilot, and leaves the potential for more patients to be exposed to an ineffective treatment). An 80% CI satisfies the need for reasonable certainty for trial decision making but is small enough to deliver a study within a reasonable budget and timeframe, although we acknowledge some people might feel more comfortable using a 90% CI. Furthermore, we used a one-sided 80% CI because we were only interested in proceeding towards a main trial if there was some evidence of effectiveness. If the intervention appeared to be harmful, even if this were not significant, it would not be reasonable to proceed.

Although our findings do not have immediate implications for clinical practice, they have implications for future clinical research. Our estimates of event rates and

effect size are promising and informative for the design of definitive clinical trials of propranolol for CCM. We have demonstrated that a multicentre clinical trial for CCM is possible in Italy, and that adherence to protocol and target doses of propranolol will require careful attention in a main phase trial. Our study has shown that a large proportion of participants can undergo serial brain MRI to monitor CCM progression as a response to treatment, which makes this a promising surrogate biomarker for future clinical trials in fCCM. The choice for an open-label design was dictated mainly by the need to perform an investigator-driven trial with limited funding. It is conceivable that performance bias could be an issue with e.g. mild focal neurological deficit, so placebo would be ideal; in fact, the Phase 2/3 trial Treat2_CCM, which has been submitted to Horizon EU for funding, will be double-blind. Another important task would be to include the much more frequent sporadic CCM and children with fCCM.

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Contributors

ED and RL had the idea for the study and acquired funding. RL supervised the study and had final responsibility for the decision to submit for publication. SL was the principal investigator of the clinical trial, wrote the original draft, and edited the manuscript. ES, JMTAM, RASS, ED, and RL reviewed and edited the manuscript. ES analysed and cured all MRI data and validated MRI examinations. JMTAM, statistician, verified the raw data before analyses. JMTAM made the statistical plan and did the statistical analyses. RP, GAB, and SL, in addition to the rest of the Steering Committee members, enrolled and followed up patients and acquired data.

Declaration of competing interests

RASS has received a National Institute of Health Research Health Technology Assessment trial grant for the Cavernomas A Randomised Effectiveness (CARE) pilot trial (Ref. NIHR128694), paid to the University of Edinburgh; consultancy fees from Recursion Pharmaceuticals, paid to the University of Edinburgh; and is on the Scientific Advisory Board for Angioma Alliance (unfunded) and is Medical Advisor & Patron for Cavernoma Alliance UK (unfunded). RL has received speaking fees from Neurelis. SL, ES, JMTAM, RP, GAB, and ED declare no competing interests.

DATA SHARING

The data are stored at the Department of Cardiovascular Medicine, Mario Negri Institute for Pharmacological Research in Milan, Italy. De-identified individual participant data, data dictionary, study protocol and informed consent form will be made available for scientific purposes on formal request and consequent approval of the proposal by the Steering Committee after publication. Please, refer to the corresponding author, Roberto Latini.

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Table 1 – Baseline demographic, clinical, and genetic characteristics of the intention-to-treat population

		All participants (n=83)	Propranolol with standard care (n=57)	Standard care (n=26)
Age (years)		45·8 ± 14·8	45·4 ± 14·2	46·8 ± 16·3
Sex (female)		48 (57·8%)	34 (59·6%)	14 (53·8%)
BMI (kg/m^2)		24·2 ± 3·8	24·3 ± 3·6	23·9 ± 4·1
Systolic BP (mmHg)		122 ± 14	122 ± 14	123 ± 13
Diastolic BP (mmHg)		78 ± 8	79 ± 9	77 ± 8
Heart rate (bpm)		71 ± 11	71·2 ± 10·7	71 ± 13
Prior ICH		48 (57·8%)	33 (57·9%)	15 (57·7%)
Prior FND		40 (48·2%)	31 (54·4%)	9 (34·6%)
Prior epileptic seizures		31 (37·3%)	21 (36·8%)	10 (38·5%)
Prior headache		59 (71·1%)	41 (71·9%)	18 (69·2%)
Genetic mutations	KRIT1	54 (65·1%)	37 (64·9%)	17 (65·4%)
	MGC4607	12 (14·5%)	7 (12·3%)	5 (19·2%)
	PDCD10	5 (6·0%)	4 (7·0%)	1 (3·8%)
	No mutation found§	12 (14·5%)	9 (15·8%)	3 (11·5%)
Hypertension		19 (22·9%)	13 (22·8%)	6 (23·1%)
Diabetes mellitus		2 (2·4%)	1 (1·8%)	1 (3·8%)
Hypercholesterolaemia		11 (13·3%)	7 (12·3%)	4 (15·4%)
Ischaemic heart disease		1 (1·2%)	0	1 (3·8%)
Antiepileptic drug treatment		35 (42·2%)	24 (42·1%)	11 (42·3%)
NSAIDs		2 (2·4%)	1 (1·8%)	1 (3·8%)
Antihypertensive treatment		20 (24·1%)	14 (24·6%)	6 (23·1%)
Antidepressant treatment		11 (13·3%)	9 (15·8%)	2 (7·7%)
Vitamin D supplementation		10 (12.0%)	8 (14.0%)	2 (7.7%)
Statin		13 (15.7%)	9 (15.8%)	4 (15.4%)

Data are represented as mean ± standard deviation, median [Q1-Q3] or N (%); BMI=body mass index; BP=blood pressure; ICH: intracerebral haemorrhage; FND=focal neurological deficit; NSAIDs=non-steroidal anti-inflammatory drugs.

§: sporadic CCM 9 patients, 1 patient was later diagnosed a leukoenkephalopathy, 1 patient with radiation-induced CCM and another patient neither had a mutation nor CCM lesions at MRI.

Table 2 – Brain MRI characteristics of CCM during 2-year follow-up by assigned treatment in 67 participants with FCCM who did not undergo neurosurgical resection of CCM and had brain MRI available

	Propranolol with standard care (n=47)			Standard care (n=20)		
	Baseline	**Year 1**	**Year 2**	**Baseline**	**Year 1**	**Year 2**
Volume of largest CCM* Independent of location, mm^3	551 [157-1621]	616 [155-1671]	616 [174-1678]	455 [94-1033]	423 [106-1060]	423 [102-1048]
Total number of supratentorial CCM per patient**	41 [16-101]	46 [16-103]	47 [18-106]	42 [20-96]	45 [20-102]	49 [21-103]
Total number of infratentorial CCM per patient **	13 [4-31]	13 [4-35]	13 [5-35]	14 [5-32]	14 [5-32]	15 [5-34]
Total number of CCM per patient	56 [21-145]	58 [21-149]	64 [23-154]	57 [24-129]	60 [24-133]	65 [25-139]
Patient with at least one Zabramski 1A CCM (extralesional bleeding)	5 (10·6%)	8 (17·0%)	7 (14·9%)	1 (5·0%)	4 (20·0%)	4 (20·0%)
Patient with at least one Zabramski 1B CCM (intralesional bleeding)	33 (70·2%)	37 (78·7%)	38 (80·9%)	9 (45·0%)	10 (50·0%)	12 (60·0%)
Patient with at least one haemorragic CCM (Zabramski 1A or 1B)	34 (72·3%)	39 (83·0%)	38 (80·9%)	9 (45·0%)	10 (50·0%)	12 (60·0%)
Patients with signs of new CCM haemorrhage compared to prior MRI	-	24 (51·1%)	28 (59·6%)	-	8 (40·0%)	9 (45·0%)

Data presented as N(%) or median [Q1-Q3].

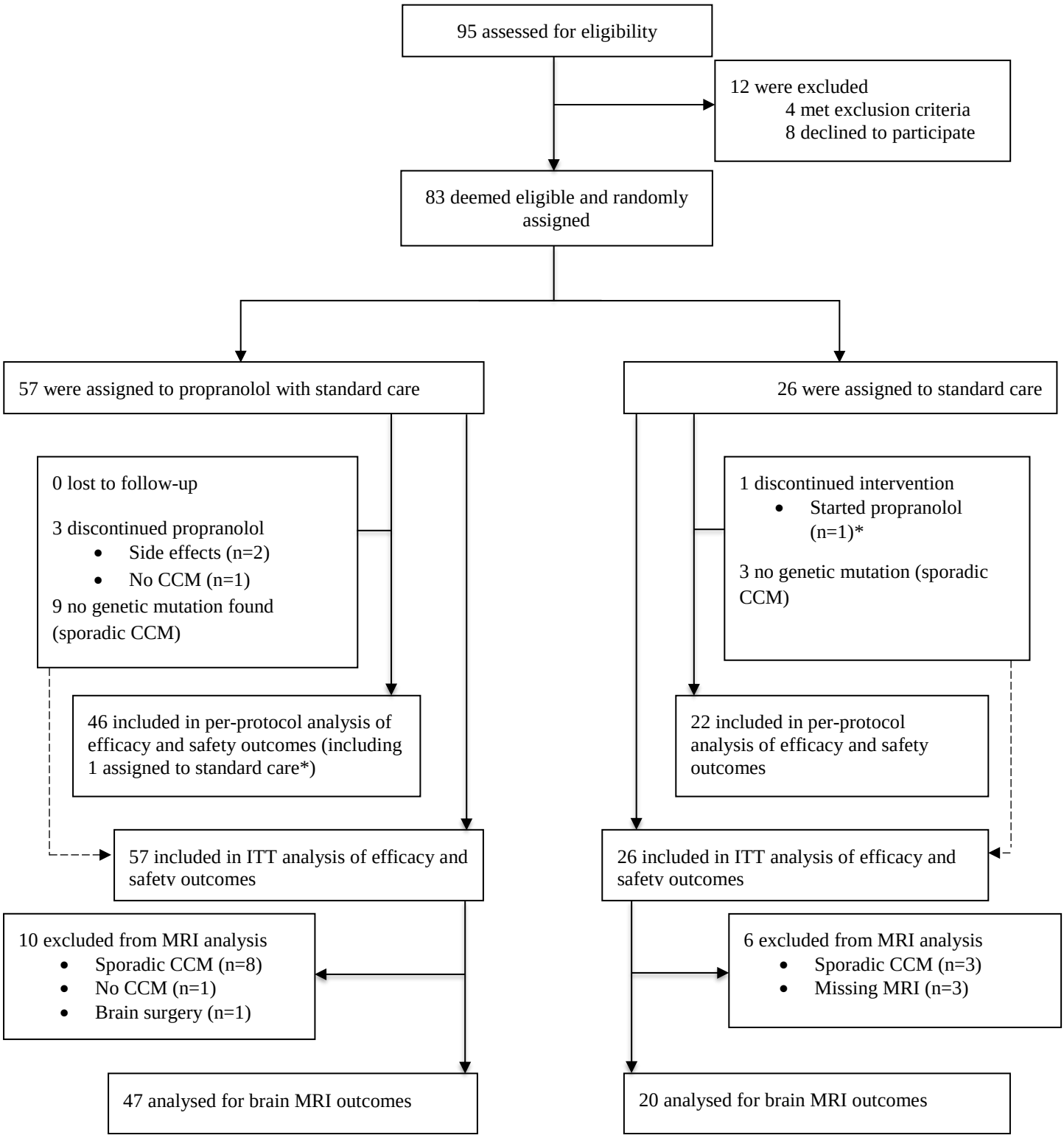
* one patient excluded from analysis for outlier for volume.

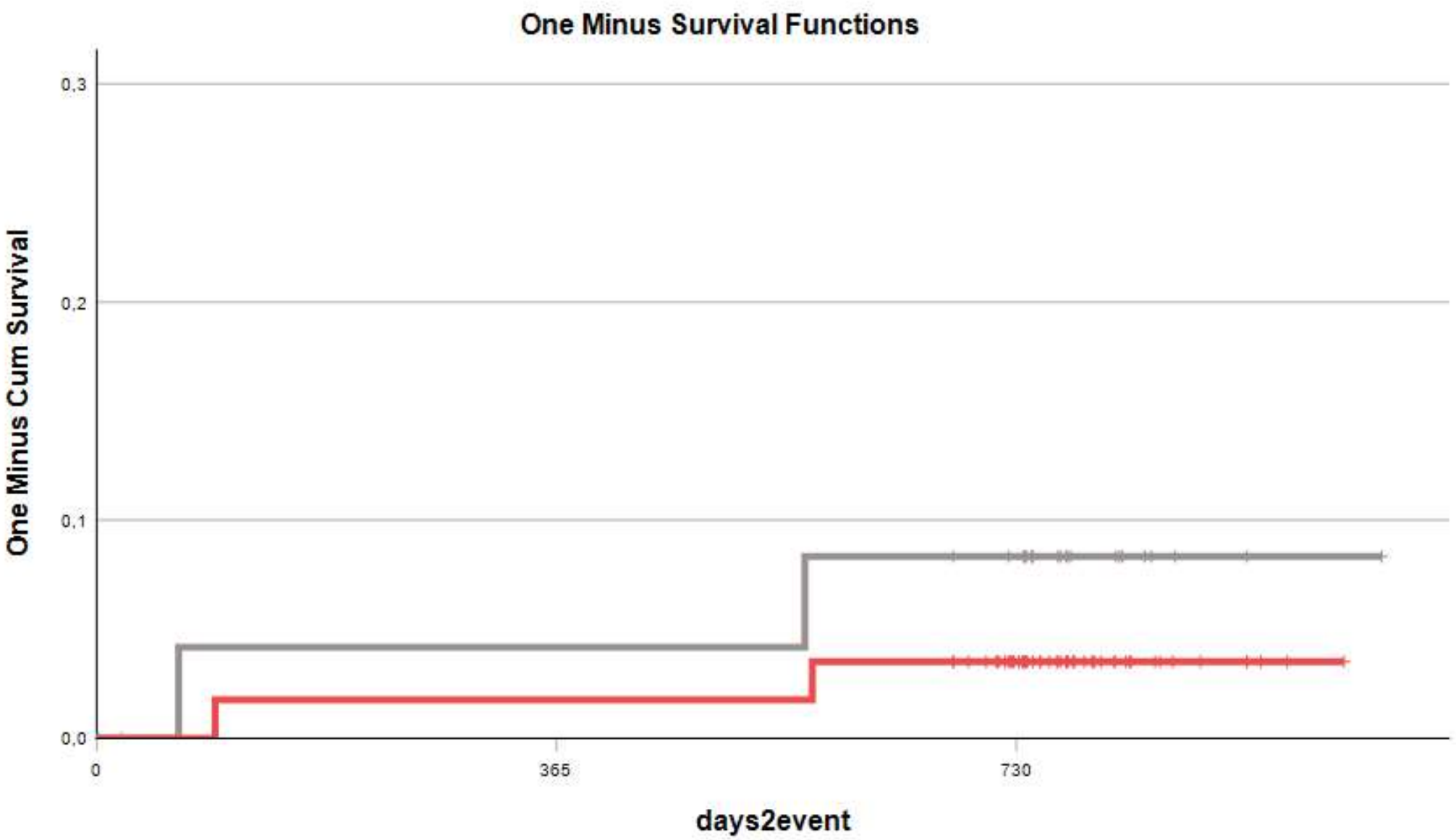
**Supra-tentorial includes the basal ganglia and cerebral hemispheres. Infra-tentorial includes cerebellum and brainstem.

Safety and efficacy of propranolol for treatment of familial cerebral cavernous malformations (Treat_CCM)

Figure 1 – Trial profile

*Included in propanol per-protocol population

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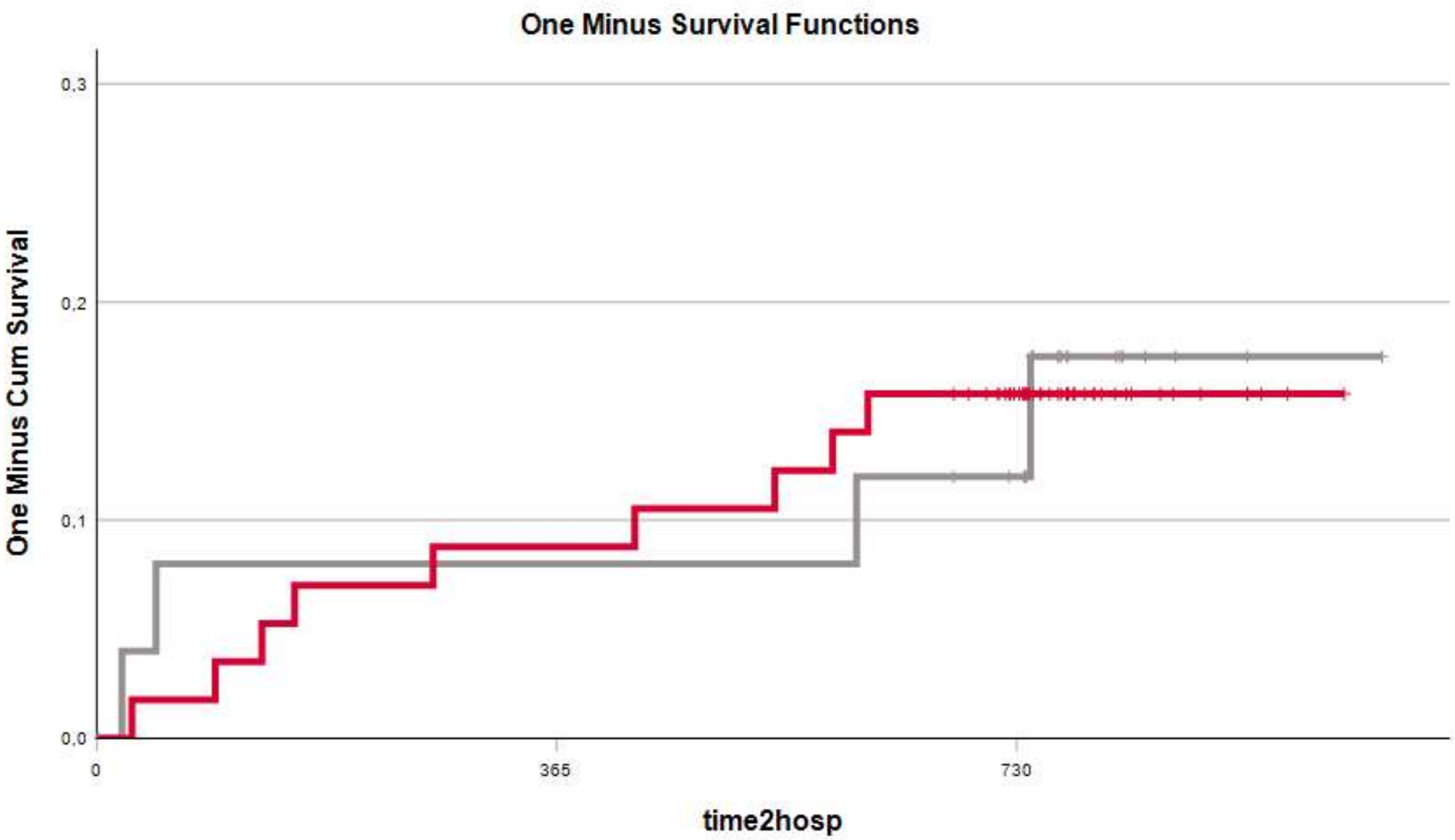
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