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Proton pump inhibitors decrease phlebotomy need in HFE hemochromatosis: double-blind randomized placebo-controlled trial

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Author involvement

Annick Vanclooster was involved in the acquisition, analysis and interpretation of data. She redacted and revised the manuscript critically, performed the statistical analysis and study supervision.

Cees van Deursen was involved in the study concept and design, acquisition, analysis and interpretation of data. He did a critical revision of the manuscript and study supervision.

Reggy Jaspers was involved in the study concept and design. She did a critical revision of the manuscript.

David Cassiman was involved in the acquisition, analysis and interpretation of data. He revised the manuscript critically, performed statistical analysis and study supervision.

Ger Koek was involved in the study concept and design, acquisition, analysis and interpretation of data. He did a critical revision of the manuscript, study supervision as well as obtaining funding.

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There was no conflict of interest for Annick Vanclooster, David Cassiman, Cees van Deursen and Reggy Jaspers

List of abbreviations

HH: Hereditary Hemochromatosis, QoL: Quality of Life, PPI: Proton pump inhibitor, SF: serum ferritin

Phlebotomy constitutes the established treatment for HFE-related hemochromatosis. Retrospective studies have suggested proton pump inhibitors (PPIs) reduce the need for phlebotomy in this population. We conducted an RCT to prove this. Thirty p.C282Y homozygous patients were randomly allocated to PPI (pantoprazole 40mg/day) or placebo for 12 months. Phlebotomies were performed when serum ferritin was > 100 µg/L. Phlebotomy need turned out to be significantly lower in patients taking PPI (P=.0052).

PPI treatment significantly reduces the need for phlebotomies in p.C282Y homozygous patients. In view of the known long-term safety profile of PPI, they can be a valuable addition to standard therapy. Clinicaltrials.gov: NCT01524757
Keywords: Hereditary Hemochromatosis, proton pump inhibitors, randomized clinical trial

Hereditary hemochromatosis (HH) is a disorder of iron homeostasis, related to mutations in the HFE-gene. The most common pathogenic mutations in the HFE-gene are the p.C282Y and p.H63D. The inheritance is autosomal recessive and the prevalence of p.C282Y homozygosity is 1/200 to 1/400 persons of Northern European descent. [1-3] Homozygous individuals may remain asymptomatic and have a normal quality of life (QoL). Iron accumulation can nevertheless cause severe organ damage, resulting in liver dysfunction (fibrosis, cirrhosis, hepatocellular carcinoma), arthropathy, diabetes mellitus [4] with impact on the QoL and survival. Complications and symptoms can be prevented or regress by reducing the body iron overload.

Established treatments to achieve iron depletion are phlebotomy, erythrocytapheresis and iron chelation. By far the most commonly applied treatment is phlebotomy. Phlebotomy is performed every week or fortnight, during the depletion phase and on average once every three to six months during the maintenance phase.[5] With each phlebotomy, about 500 ml of total blood, corresponding to roughly 250 mg of iron, is removed.

Side effects of phlebotomy (fatigue, fainting, loss of appetite) are experienced by 52% of the patients in the induction phase, and by 37% in the maintenance phase.[6] Therefore, alternative treatments are warranted.

Two publications report on the effect of proton pump inhibitor (PPI) in patients with HH. An observational study reported a reduction from 5 to 1 phlebotomy per year in seven p.C282Y homozygous HH patients on PPI treatment.[7] A recent retrospective study confirmed the significant reduction in phlebotomies from a median of 3.17 before PPI treatment to 0.5 per year with PPI, in 57 p.C282Y homozygous HH patients. [8]

These two observational, non-randomized studies suggest that HH patients on PPI need fewer phlebotomies. To confirm these potentially important observations, we initiated a prospective

randomized double-blind, placebo-controlled study in homozygous p.C282Y HH patients. We hypothesized that the use of PPI would significantly reduce the need for phlebotomies.

Fifteen patients were assigned to each group. Compliance in both groups was comparable with an average of >90% drug intake. Baseline demographics and clinical characteristics of the included patients are detailed in table 1.

A total of 31 patients were randomly enrolled either to the placebo or the PPI (pantoprazol 40 mg per day) group. One patient terminated the study after two months due to a facial rash (placebo group). The data of this patient were not included in the final results. One patient withdrew from the study due to fatigue after 10 months (PPI group). The results of this patient were included until withdrawal.

At diagnosis, the HH patients had an average serum ferritin (SF) level of 1414 ± 867 µg/L (mean ± SD). Complaints at inclusion were fatigue in 44.8% (13/29), joint complaints in 53.3% (16/30) and sexual dysfunction in 36% (9/25).

There was no significant difference between the two groups for age neither at diagnosis nor at start of the study and also for the number of phlebotomies needed per year before start of the study (Table 1). However, a significant difference (P=.0052) was measured in the total number of phlebotomies needed during the study period (Table 1; Figure 1). Patient 9 (placebo) didn't need any phlebotomy during study period, while 4 respectively 2 in the two years before.

No serious side-effects were registered. One patient (PPI) experienced diarrhea for three days, due to a Campylobacter infection. The patient described more fatigue at the end of the study. Two patients (placebo) experienced an unpleasant feeling in the stomach. One patient (placebo) was more fatigued after the twelve months' study time. Increasing arthralgia was described in three patients (two placebo, 1 PPI).

One patient (PPI) withdrew after 10 months because of fatigue, one patient (placebo) had transient increased liver tests during an episode of viral upper respiratory infection and one patient (PPI) had increased liver tests confirmed to be steatohepatitis.

Iron and biochemical variables

Although the SF level in the PPI group turned out to be significantly higher at the start (randomization was not based on SF levels), this group reached a significantly lower SF level after the twelve months of treatment (P=.0145), despite a significantly lower number of phlebotomies (Table 1). The serum gastrin levels were significantly higher in the PPI group at the end of the study period, consistent with the use of PPI. Transaminase levels and vitamin B12 levels did not change significantly (data not shown).

The daily use of PPI significantly reduced the number of phlebotomies needed to keep the ferritin level < 100 µg/L in HH patients homozygous for p.C282Y. This is the first randomized controlled trial (RCT) with PPI in HH patients to demonstrate that effect. The study confirms the results of the observational, retrospective studies by Hutchinson and van Aerts.[7,8]

An important concern in long-term treatment with PPI, is the development of side-effects.[9] In our study, no serious adverse events were encountered during the one-year study period. Freedberg et al.[10] stated in their recent review that the list of potential adverse effects associated with PPI use is long, but the absolute risk increase is modest and the quality of evidence is low to very low.

Our study shows a few limitations. The number of included patients (n=30) was lower than the number based on the power calculation previous to initiation (i.e. 48). There was a stagnation of inclusions at 31, mostly due to the stringent in- and exclusion criteria (e.g. PPI use). However, the number of patients that participated, proved sufficiently large to achieve

significance, as the measured PPI effect exceeded the effect we projected. The consumption of tea was not recorded before and during the trial. [11]

This RCT proves that a significant reduction in phlebotomy need in p.C282Y homozygous hemochromatosis patients can be achieved by treating them with PPI. The results of this study open the way to individualize treatment for p.C282Y homozygous patients. Future research must unravel the mechanisms, which will help to target PPI therapy more precisely. Studies should focus on the minimal effective dose, long term side effects, QoL and cost.

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Figure legend

Figure 1: Total number of phlebotomies during the 12 months before the trial and during the 12 months of the trial

Table 1: Patient characteristics and clinical outcome parameters

	Placebo (n=15)	PPI (n=15)	P-value
Age (years as mean with SD)			
At diagnosis	47.13 (± 11.04)	50.13 (± 8.86)	.2
Start of study	53 (± 10.5)	57.53 (± 6.51)	.49
Sex			
Male	12 (80%)	10 (67%)	
BMI	27.8 (± 3.39)	27.05 (± 4.37)	.3
Phlebotomies			
Year before study	4.87 (± 1.60)	5.33 (± 1.63)	.21
During study time	2.60 (± 1.55)	1.27 (± 1.03)	**.0052**
Ferritin level (U/L)			
0 months	57.53 (± 10.02)	74.40 (± 27.55)	**.039**
6 months	87.80 (± 19.25)	81.73 (± 24.54)	.22
12 months	125.80 (± 37.06)	90.53 (± 46.18)	**.0145**
Transferrin Saturation (%)			
0 months	51.13 (± 17.01)	42.40 (± 16.40)	.082
6 months	62.93 (± 17.68)	50.79 (± 19.01)	**.043**
12 months	61.79 (± 17.94)	55.23 (± 24.15)	.217
Gastrin level (pg/mL)			
0 months	36.47 (± 24.15)	56.13 (± 73.74)	.170
12 months	32.73 (± 17.91)‡	96.64 (± 71)‡	**.0071**

BMI: Body Mass index; ‡ 13 patients.

SF level at inclusion was set between 50-100 µg/L, there was a slight deviation in 33% (39 – 138 µg/L) of the patients.

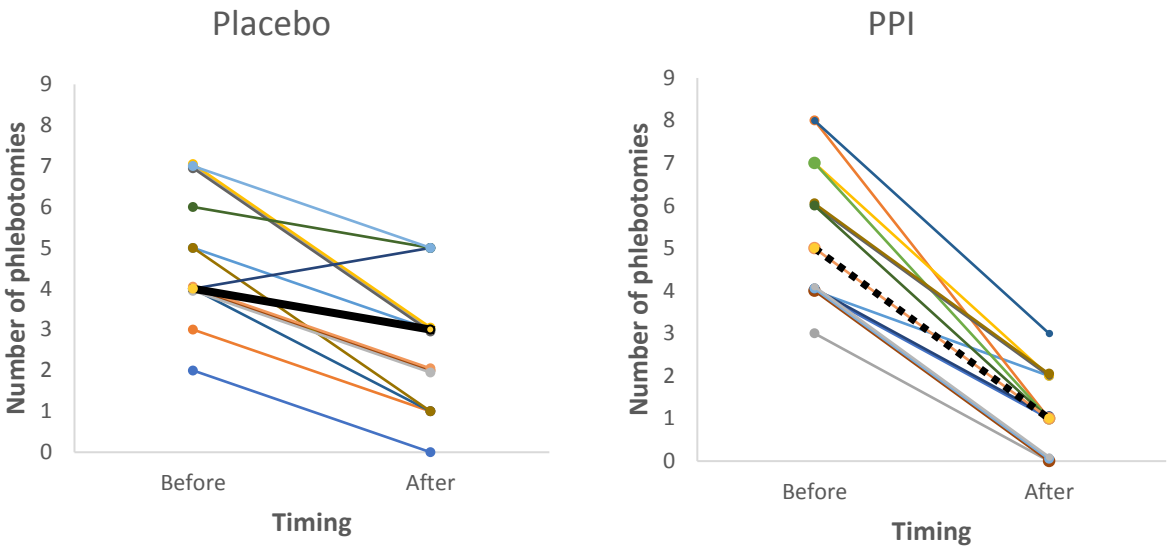

Figure 1: Total number of phlebotomies during the 12 months before the trial and during the 12 months of the trial. ▬▬▬ = median

Study design

A randomized double-blind placebo-controlled trial was performed, during a trial period of 12 months, with pantoprazol 40 mg as the PPI. Via computer-generated randomization, the hospital pharmacist randomized, by block size of 6, patients to the placebo or PPI group. The code was kept at the pharmacy of both participating hospitals. All tablets, PPI as well as placebo, were manufactured by Takeda GmbH, Plant Oranienburg, Germany and had the same appearance and taste. PPIs and placebo's were delivered in bulk, repackaged and labeled. Study participants as well as involved researchers were blinded during the entire study. At the end of the study, the randomization code was provided by the hospital pharmacist to the care providers via e-mail.

After randomization, patients underwent routine laboratory evaluation with liver enzymes: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma glutamyltransferase, serum magnesium level, iron, transferrin and transferrin saturation. Hematological parameters measured were hemoglobin, white blood cell count and platelets, at baseline and every six months. Vitamin B12 and gastrin concentrations were measured at baseline and at 12 months of treatment. Serum ferritin (SF) level was measured at baseline and every two months using the

ElectroChemiLuminescense Immuno Assay (ECLIA; sandwich assay) of Roche Diagnostics. If the SF was > 100 µg/L, a phlebotomy of 500 ml whole blood was performed.

Study Participants

Inclusion and exclusion criteria are described below. If an indication to start PPIs would arise during the study period, the patient was planned to be withdrawn from the study.

Participants were included from September 2013 till July 2014. The study ended in July 2015, when the last patient ended the 12 months study period. All participants gave written informed consent. Study participants received their medication from their physician and compliance was measured by counting the number of returned tablets. Compliance was

Inclusion criteria	Exclusion criteria
Homozygous p. C282Y mutation	Chelating therapy
Maintenance therapy for at least 12 months	Forced dietary regimen
≥ three phlebotomies/year	Mentally incapable
SF level: 50-100 µg/L	Women being pregnant or planning to become pregnant
Age: 18-75 years	Patients with malignancy

calculated by counting the days between two visits and the returned PPI/placebo tablets.

Weight: > 50 kg	BMI: ≥ 35kg/m^2
	On PPI treatment or earlier side effects

SF: Serum ferritin; BMI: Body Mass Index; PPI: Proton Pump Inhibitor

The sample size was calculated with a power of 90% and α of 0.05 for a pre-specified relevant reduction in number of yearly phlebotomies from 5 to 2 (Standard deviation (SD) 3), the calculated number of patients to be included was 48. In total, 31 patients entered the trial.

Patient involvement

Before starting the study, patients were informed about the design of the study during the organization of patient days organized by two patients organizations (Hemochromatose Vereniging Nederland, the Netherlands and Haemochromatose Vereniging Vlaanderen, Belgium). Prior to randomization, all assessments were discussed with the patients in a face-to-face meeting. Patients who were involved in the study were informed personally about the results of themselves and of the entire study. During and after the study, we thanked the patients for their interest and involvement.

Study setting

The study protocol was compliant with the ethical guidelines of the 1975 Declaration of Helsinki. Two hospitals were involved: the Zuyderland Medical Center in Heerlen, the Netherlands and the University Hospital Leuven (UHL), Gasthuisberg, Belgium. This study was registered in a public register (NCT01524757) and an approval of both ethical committees was received (UHL: EudraCT Number 2012-000603-32; ML9419 – Zuyderland Medical Center in Heerlen: NL33544.096.12, METC 12-T-04).

Statistical analysis

Statistical analyses were performed by using R-studio.[15] A two-tailed independent sample t-test was used to verify whether the means of continuous measurements were the same in the two independent groups. A p-value equal or less than 0.05 was considered significant.

Data from the two different study groups, PPI and placebo, were pooled and put off against each other.

All authors had access to the study data, reviewed and approved the final manuscript.

Side-effects

No serious side-effects in the intervention or placebo group were registered. One patient experienced diarrhea for three days, occurring after one month of treatment, which turned out to be a Campylobacter infection. The same patient described more fatigue at the end of the study. After deblinding, this patient was in the PPI group and needed three phlebotomies in total. Two patients described an unpleasant feeling in the stomach. One patient, taking placebo, was also more fatigued after twelve months study time and three phlebotomies. Increasing arthralgia was described in three patients (two placebo, 1 PPI group).

One patient withdrew after 10 months because of fatigue (PPI group), one patient had transient increased liver tests during an episode of viral upper airway infection (placebo group) and one patient had increased liver tests suggested to be steatohepatitis confirmed by sonography (PPI group). The magnesium and vitamin B12 concentrations remained in the normal range in all patients.