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A Vancomycin-induced shift of the gut microbiome in gram negative direction increases plasma Factor VIII:C levels: Results from a randomized, controlled trial

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Abstract

Background: The gut microbiome might be a source of systemic inflammation and activation of coagulation by translocation of lipopolysaccharides from gram negative bacteria to the systemic circulation.

Objective: To investigate whether a Vancomycin-induced shift of the gut microbiome in a gram negative direction influences systemic inflammation and plasma factor (F) VIII procoagulant activity (FVIII:C).

Design: Randomized, single (investigator) blinded, controlled trial

Setting: Single-center trial in Norway

Participants: 50 healthy volunteers were screened and randomized, and 43 participants aged 19-37 years completed the trial.

Intervention: Oral Vancomycin 500 mg t.i.d. for seven days or no intervention (1:1)

Measurements: Feces and blood were sampled at baseline, the day after the end of intervention , and three weeks later. Gut microbiome composition was assessed by a phylogenetic assay. Cytokines were measured using multiplex technology, complement activation was measured using ELISA, FVIII:C was measured using an APTT-based assay, and high-sensitivity (hs)CRP was measured by an immunoturbidimetric assay.

Results: Twenty-one subjects were randomized to intervention and 22 subjects served as controls. Vancomycin intake reduced gut microbiome diversity and increased the abundance of gram negative bacteria. FVIII:C and in hs-CRP increased significantly in the intervention group compared to the control group. The cytokines and complement activation markers were similar in the two groups.

Limitations: Potential effects of intervention in the range below the lower assay detection limits could not be excluded. Post-hoc power calculation revealed study power of 70% for the main outcome FVIII:C.

Conclusion: A Vancomycin-induced change in the composition of the gut microbiome in a gram negative direction was accompanied by significant increase in plasma FVIII:C and hs-CRP levels.

Registration: trialregister.nl, NTR-TC: 5093

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Introduction

Venous thromboembolism (VTE) is a common and complex cardiovascular disease with an annual incidence of 1-2 per 1000 in the adult population (1). VTE is associated with severe short- and long-term complications, including death (1). In contrast to arterial cardiovascular diseases, like myocardial infarction and stroke (2, 3), the incidence of VTE is not declining (4).

VTE is a multicausal disease, and inflammation is a shared feature in several conditions associated with risk of VTE, including cancer (5, 6), obesity (7-9), inflammatory bowel disease (IBD) (10), infections and antibiotic use (11-14). In addition, these conditions are also characterized by a dysbiosis of the gut microbiome. The gut microbiome in healthy adults is dominated by two bacterial phyla, the *Bacteroidetes* (consisting of gram negative bacteria), and the *Firmicutes* (consisting of mainly gram positive bacteria) (15). A healthy gut microbiome has been associated with high diversity (16), whereas a less diverse composition of the gut microbiome has been demonstrated in obesity (17), IBD (18), *Clostridium difficile*-infection (19) and in cancer (20). However, many, yet to be unraveled, underlying complex mechanisms and interactions contribute to this, and the term "dysbiosis" reflects an unbalance in the relationship between the gut microbiome and the host.

Inflammation and coagulation are closely linked through interactions between the complement system, the coagulation- and fibrinolytic systems (21, 22), and through interactions with these systems and the pro-inflammatory cytokine network (22). Gut microbiome dysbiosis may potentially affect coagulation through low-grade inflammation driven by lipopolysaccharides (LPS) from gram negative bacteria translocated from the gut to the systemic circulation (23-25). Coagulation factor (F) VIII plays a central role in the coagulation cascade as a co-factor for activated FIX in the intrinsic pathway (26). Procoagulant FVIII activity (FVIII:C) is a predictor of both first (27) and recurrent (28) VTE, and is also an acute phase reactant (29). Other inflammatory markers, such as high-sensitivity C-reactive protein (hs-CRP) have not been associated with VTE risk in studies with long-term follow-up (30, 31), but prospective cohorts with shorter follow-up time have shown an

association between hs-CRP and VTE (32, 33). Using repeated measurements, we found that high levels of CRP (≥3 mg/L versus <1 mg/L) were associated with a 1.8-fold increased risk of VTE in women (HR 1.84, 95% CI 1.22-2.78), but not in men after 3.1 years of follow-up in the Tromsø Study (34). Inflammation seemed to play a role for VTE risk in obese women, as adjustment for CRP attenuated the risk estimate for VTE by 22% (34).

On this background, we hypothesized that a dysbiosis of the gut microbiome may elicit a systemic inflammatory response, which in turn can increase plasma FVIII:C levels, and thereby explain some of the VTE risk observed in conditions such as obesity, cancer and IBD. To investigate this hypothesis, we performed a randomized, controlled trial in healthy volunteers, to explore whether a shift in the gut microbiome in a gram negative direction influences systemic inflammation and plasma FVIII:C levels.

Methods

Participants and trial design

This randomized, controlled, investigator-blinded trial had a double-arm parallel design, and 21 participants were recruited to the intervention group and 22 to the control group. Previous studies have reported differences in the gut microbiome composition in lean and overweight/obese subjects. To allow for subgroup analysis, we recruited a minimum of 10 lean (BMI <25 kg/m^2) and 10 overweight/obese (BMI ≥25 kg/m^2) subjects to both the intervention and the control group. No changes to methods were applied after trial commencement.

Exclusion criteria were the use of antibiotics for a period of 2 months or the use of any medication except oral contraceptives in the last month before entering the study, pregnancy or post-partum state, chronic inflammatory conditions (IBD, rheumatic disease, diabetes mellitus, HIV-infection), gastro-intestinal disease in the last month before entering the study, a history of irritable

bowel disease, allergy for Vancomycin or Teicoplanin, liver or kidney disease, a feverish episode the last two weeks, planned diet change, the start of systematic use of probiotics during the last two months, and travel outside of Europe the last month before entering the study. Liver and kidney function were evaluated by s-Alanine Aminotransferase, s-Alkaline phosphatase and s-Creatinine, and pregnancy testing was performed by serum (s)-Human Chorionic Gonadotropin (HCG) in women before study start.

Subjects were recruited via advertisements at UiT - the Arctic University of Norway in Tromsø, Norway. The study took place at the Clinical Research Unit at the University Hospital of North Norway. Volunteers contacted the study doctor by e-mail for written information about the study, and those still interested were invited for a screening/information visit with the study doctor. Eligible subjects who signed a written consent were randomized and enrolled for three study visits where blood and feces samples were collected. The randomization was performed by the central randomization unit at the University Hospital of North Norway through block randomization (block sizes of 2 and 4) with stratification for BMI. Detailed information on recruitment is provided in Figure 1 (Flow diagram).

Intervention

Participants were randomized to oral intake of Vancomycin, 500 mg (4 capsules of 125 mg) three times per day for seven consecutive days (the intervention group) or to no intervention (the control group). Vancomycin is effective against gram positive bacteria (35), and has in a trial on insulin sensitivity been shown to provide the desired gram negative shift of the microbiome using identical doses and duration of intervention (36). The participants were asked to return capsules and drug packaging, and returned capsules were counted to assess compliance. All participants were asked to maintain their habitual physical activity pattern and dietary habits throughout the study. Hard physical activity and alcohol intake the day before blood sampling were discouraged. At baseline (T0), the day after the intervention stopped (T1), and three weeks after end of intervention (T2),

blood was sampled after a normal breakfast and feces samples, produced within the last 24 hours, were collected.

Outcomes

The predefined primary outcome was the difference in change (between T0 and T1) of coagulation FVIII:C between the intervention and the control group. Secondary outcomes were inflammatory parameters including hs-CRP, fibrinogen, interleukin (IL)-1β, IL-6, IL-8, IL-10, monocyte chemoattractant protein (MCP)-1, neutrophil cell count and tumor necrosis factor (TNF). Complement activation products were assessed by C3bc, reflecting the proximal complement pathway, and terminal complement complex (TCC). Gut microbiome composition was assessed to ensure that the intervention actually did change the balance between gram positive and gram negative bacteria. All outcomes were assessed at T0, T1 and T2.

Sample size calculation

The sample size was determined on the primary outcome, i.e. FVIII:C, based on an estimated effect similar to that observed in treatment-naïve patients with HIV-infection (37). Treatment-naïve HIV-patients had mean FVIII:C levels of 222 IU/dL (standard deviation [SD] 11), whereas controls had mean FVIII:C levels of 100 IU/dL (SD 11). With an alpha of 5% and 20 participants in both study arms, we would have >99% power to detect the same difference in FVIII:C as observed in the HIV-study.

Laboratory analyses of blood

Blood was collected from an antecubital vein. Neutrophil cell count, fibrinogen and hs-CRP were analyzed at the Department of Clinical Biochemistry at UNN within few hours after sampling. Neutrophil cell count was analyzed in EDTA-blood on an automated blood cell counter by a fluorescence flow-cytometric method (Sysmex XN, Sysmex Nordic ApS), with a coefficient of variation (CV) of <5%. Fibrinogen was analyzed in plasma prepared by centrifugation of sodium-citrated blood at 2500 x *g* for 15 minutes, and analyzed by a clotting method (STA® -Liquid Fib, STA-R Evolution,

Diagnostica Stago, France), with a CV of <5%. Hs-CRP was analyzed in serum prepared by centrifugation at 2000 x *g* for 15 minutes of blood sampled on a serum-separating tube, by an immunoturbidimetric assay on a Cobas 8000 autoanalyzer (Roche Hitachi, Mannheim, Germany). The CV for hs-CRP was 2.9% at CRP-level 1.65 mg/L, and 4.45% for all CRP-levels. The cytokines IL-1β, IL-6, IL-8, IL-10, MCP-1, TNF, and complement activation products (C3bc and TCC) were analyzed in EDTA-plasma prepared immediately after blood sampling. Whole blood was placed on crushed ice, centrifuged within 30 minutes at 2500 x *g* at 4°C for 15 minutes, and then stored at -70°C. Plasma concentrations of the cytokines were determined using a multiplex immunoassay system (Bio-Plex® Multiplex System, Bio-Rad Laboratories, Inc. Hercules, CA, USA). Values for IL-1β, IL-8, IL-10 and MCP-1 reported below the lower detection limit were set at the lowest reported value. The complement activation products were analyzed using ELISA. Analyses of FVIII:C was performed in plasma prepared from blood samples containing sodium citrate as anticoagulant by centrifugation in two steps, first at 2500 x *g* for 15 minutes, and then at 10 000 x *g* for 10 minutes. Plasma was stored in cryovials at -70˚C until analysis. FVIII:C was measured using an ACL TOP 300 CTS® (Instrumentation Laboratory, MA, USA) with SynthAsil APTT (Instrumentation Laboratory Werfen, New Delhi, India) reagents in FVIII deficient citrated plasma.

Laboratory analysis of gut microbiome

DNA was extracted from fecal material using a repeated bead beating (RBB) protocol (38). DNA was purified using Maxwell RSC Whole Blood DNA Kit. 16S rRNA gene amplicons were generated using a single step PCR protocol targeting the V3-V4 region (39). PCR products were purified using Ampure XP beads and purified products were equimolar pooled. The libraries were sequenced using a MiSeq platform using V3 chemistry with 2x251 cycles. Data was submitted to ENA repository under study PRJEB25759. Forward and reverse reads were length trimmed at 240 and 210 respectively and ASVs were inferred and merged using dada2 (V1.5.2) (40). Taxonomy was assigned using the RDP classifier and SILVA 16S ribosomal database V128 (40). Microbiota data was further analyzed and visualized

using phyloseq (41), vegan (https://cran.r-project.org/web/packages/vegan/index.html/) and picante (42) packages.

Statistical analyses

Statistical analyses of all non-feces data were carried out using STATA version 14.0 (Stata Corporation, College Station, Texas, USA) and R version 3.4.3 (https://www.R-project.org). All data were evaluated for normality and outliers by inspection of box plots. One outlier in the C3bc-variable and one in the TNF variable were detected, and values at T0, T1 and T2 in these cases were set as missing. Both subjects with outlier values belonged to the control group. Normal distribution was present for all variables except hs-CRP, and to achieve normal distribution, values for hs-CRP were logarithmic transformed for use in statistical analyses. Data were presented by means ± standard deviations (SD). Differences between the intervention and the control group in change from baseline to after intervention were tested with a two-sample, two-sided t-test. In case of significant results in the primary analyses, the analyses were further performed in the BMI-subgroups.

Statistical analysis of the gut microbiome was performed using R version 3.4.4. Differential abundance of the taxa at genus level was tested using DESeq2 (43). Differences in alpha diversity were tested using Mann-Whitney U Test. For reproduction of the results, additional details and data can be found at https://github.com/AMCMC/Project_GRIEG.

Study Oversight

The study was approved by the Regional Committee for Medical and Health Research Ethics (2015/597/REK Nord) and the Norwegian Data Inspectorate.

Role of the Funding source

The trial was funded by an independent grant from Stiftelsen K. G. Jebsen. The investigators designed the trial, conducted the protocol, and wrote the manuscript.

Results

The study was conducted from Sept 15, 2016 until J une 1, 2017. Fifty healthy volunteers aged 19-37 years were included in the study, and after withdrawals and exclusions, 43 (29 women and 14 men) completed the study and were included in our analyses (Figure 1). Baseline characteristics are shown in Table 1. There were no serious adverse events. Three participants experienced mild diarrhea of short duration during intervention with Vancomycin.

The gut microbiome composition in the intervention and the control groups at T0, T1 and T2 is shown in Figure 2. Following intervention with Vancomycin, the gut microbiome was less diverse ($p < 10^{-8}$) and had a relative increase in gram negative bacteria as compared to gram positive bacteria. This was largely due to a reduction in *Firmicutes* and other gram positive bacteria ($p < 10^{-11}$). At T1, the subgroup with BMI <25 kg/m^2 had relatively more gram negative bacteria belonging to the *Escherichia coli/Shigella*-group than the subgroup with BMI ≥25 kg/m^2 (p= 0.076). At T2, three weeks after the intervention was stopped, the gut microbiome became again more diverse, although still slightly different compared to baseline. In controls, the gut microbiome remained stable throughout the entire study period.

Results of inflammation parameters and FVIII:C at baseline (T0) and after seven days of oral Vancomycin intake (T1) in the intervention (I) group and at corresponding time points in the control (C) group are shown in Table 2. There was a significant increase in FVIII:C in the intervention group when compared to the control group (ΔI: FVIII:C= 4 IU/dL *vs* ΔC: FVIII:C= -6 IU/dL, p=0.01) (Figure 3 and 4). In the predefined subgroup analysis, this difference between the groups was present only in those with BMI <25 kg/m^2 (ΔI: FVIII:C= 6 IU/dL *vs* ΔC: FVIII:C= -7 IU/dL, p=0.04), and not in those with BMI ≥25 kg/m^2. Similar results were obtained for log transformed CRP (ΔI: logCRP= 0.21 *vs* ΔC: logCRP= -0.25, p= 0.04) (Figure 3 and 4). In the subgroup analysis, this difference was solely present in the BMI <25 kg/m^2-group (ΔI: logCRP= 0.32 *vs* ΔC: logCRP= -0.55, p= 0.02). The change from T0 to T1 in IL-6 was slightly higher, although not statistically significant, in the intervention group than in

the control group (ΔI: IL-6= 0.1 pg/L *vs* ΔC: IL-6= -0.3 pg/L, p=0.10) (Figure 3). The plasma levels of FVIII:C, CRP and IL-6 decreased from the day after end of Vancomycin treatment (T1) to the last study visit (T2) (Figure 3).

For the other inflammatory variables, i.e. neutrophil count, fibrinogen, the cytokines IL-1β, IL-8, IL-10, MCP-1 and TNF, and the complement activation products C3bc and TCC, changes in levels from T0 to T1 did not differ between the intervention and the control group (Table 2).

Discussion

Intake of oral Vancomycin resulted in a switch in the gut microbiome yielding a decrease of total phylogenetic diversity, and an increase in the proportion of gram negative bacteria at the expense of a decrease in the proportion of gram positive bacteria. Our hypothesis was that this predicted change towards a less diverse and more gram negative gut microbiome, would lead to increased systemic inflammation and higher plasma levels of FVIII:C. In line with our hypothesis, we observed a statistically significant increase in hs-CRP and FVIII:C following Vancomycin intervention, compared to the control group.

These outcomes were mainly driven by the effect of our intervention in the lean BMI group. As illustrated in Figure 2, the gut microbiome of the lean volunteers consisted of relatively more *Escheria Coli/Shigella* species compared to the overweight/obese volunteers after intervention, albeit not statistically significant. These gram negative species are in general highly associated with pathology in humans and might be causally involved in our findings of significantly increased systemic inflammation and levels of FVIII:C in the lean intervention group.

FVIII:C is an essential co-factor in the coagulation cascade and an acute phase reactant that has consistently been associated with an independent risk of both incident and recurrent VTE (44). In our study, we found a small, though significant, increase in FVIII:C after a shift of the gut microbiome in gram negative direction. Although no cohort study has investigated the impact of

FVIII:C levels on future risk of venous thromboembolism (VTE), results from several case-control studies point towards a dose-response relationship (44). FVIII:C levels increase with age (45) and demonstrate significant inter-individual variation (44). Thus, in individuals with either an age-related or a genetically higher baseline level of FVIII:C than observed in our trial, even a small increase in FVIII:C might contribute to VTE risk.

CRP is regarded as a sensitive, dynamic and quickly reacting acute-phase protein (46) and is a downstream marker of inflammation produced in the liver in response to IL-6 and other cytokines (46, 47). Therefore, serum levels of CRP may be considered the resultant marker of systemic inflammation induced by complement activation and several cytokines operating both as a cascade and as a network (47). Stimulation of CRP production from several cytokines could potentially explain why CRP and not IL-6 reached statistically significant differences. Further, activation of complement and release of cytokines may well occur locally without being reflected in the systemic circulation, due to the large dilution and short half-life of these mediators.

While CRP in healthy individuals show relatively little biological variability (90% of healthy adults have CRP <3mg/L) (46), larger biological variability exists for pro-inflammatory cytokines. Also, in our young and healthy participants, several individuals had cytokine levels below the lower detection limit of the assays used in our study, thereby camouflaging possible increases within this low range. Taking all this into account, our study was still able to show a significant increase in hs-CRP after intervention with Vancomycin, compared to the control group. This supports our hypothesis that a gram negative shift of the gut microbiome slightly increases systemic inflammation.

Acute, or at least more short-term inflammation, seems to play a greater role for VTE risk than long-term, chronic inflammation. In a recent meta-analysis of cohort studies on CRP and VTE risk, pooled estimates were consistent with a linear dose-response relationship, characterized by a 23% increase in VTE risk for every 5 mg/L increase in CRP levels (48). In our study, a shift towards a gram negative microbiome led to a significant increase in hs-CRP, probably too small to exert an

effect on VTE risk in our population of young and healthy volunteers. However, in older or less healthy people, with additional risk factors for VTE and probably higher inflammatory activity, even small increases in systemic inflammation might contribute to exceed the thrombosis threshold (49).

Another recent study investigated inflammatory markers after an intervention with antibiotics in humans. In this randomized controlled trial, Reijnders and colleagues explored the effects of Vancomycin and Amoxicillin (a broad-spectrum antibiotic) on the gut microbiome and inflammatory parameters in overweight and obese men (50). As expected, they found a shift in the microbiome composition in a gram negative direction in the Vancomycin group, but not in the Amoxicillin group. This change did not affect the concentrations of IL-6, IL-8 or TNF when compared to the placebo group. Unfortunately, CRP was not analyzed. They found no increase in gut permeability or concentration of LPS-binding protein following Vancomycin treatment (50).

Bacterial and endotoxin translocation in the gut (metabolic endotoxemia) occurs in healthy individuals at a baseline rate of 5-10% (24, 51). Even with an expected low baseline rate of bacterial translocation in our young and healthy trial participants , we were able to show significant increases in FVIII:C and hs-CRP in the intervention group compared to the control group.

Our study has both strengths and limitations. The randomized, controlled trial design is a clear strength. As CRP has shown stronger association with VTE risk in women than in men (34), the inclusion of proportionally more women might be a strength. On the other side, CRP-levels may fluctuate during the menstrual cycle (52, 53). In the present study, the number of female participants was similar in the intervention and the control groups and we would expect women in various stages of their menstrual cycle to be randomly assigned to each group. A systematic review found that FVIII:C do not show cyclic variation (54). The inclusion of young and healthy participants reduced the risk of outcomes being influenced by other factors than the intervention. The effect on inflammation and FVIII:C might also be limited for the same reason, and in subjects with higher rate of bacterial translocation, a more pronounced effect on inflammation and FVIII:C might be expected. We

hypothesized that an effect on inflammation by a gram negative shift of the gut microbiome would be mediated by translocation of LPS from the gut to the systemic circulation, and systemic LPS-levels should ideally have been measured. However, sensitive and reliable methods for measuring low levels of LPS are unfortunately not available. Consequently, we cannot verify that the observed effect was due to LPS-translocation, and an interplay between other microbes or microbial products and the host cannot be ruled out.

Our initial power calculations were based on observations in HIV patients. However, the effect on FVIII:C following Vancomycin intervention was smaller than that observed in the study of HIV-patients (37). Post-hoc power analysis based on the observed mean values with standard deviations for change in FVIII:C from T0 to T1, revealed that our study had a power of 70% with a significance level of 5%.

In conclusion, this randomized controlled trial in healthy volunteers supports the hypothesis that a Vancomycin-induced decreased gut microbiome diversity and increased gram negative composition results in increased systemic inflammation, measured by hs-CRP, and FVIII:C. Future studies are warranted to investigate the relationship between the gut microbiome, inflammation and coagulation in subjects with a higher rate of bacterial translocation.

Contributions: GG and VT conceived the idea of the study. VT and JBH obtained funding for the study. GG, VT, and JBH designed the study. GG and VT wrote the protocol and obtained approval of the Regional Ethics Committee and the Clinical Research Unit at the University Hospital of North Norway. GG ran the trial as principal investigator, included the volunteers and collected the data. GG and KH performed statistical analysis on the inflammatory and coagulation outcomes. MD performed statistical analysis of the microbiome data. MD, VT and GG interpreted the results of the microbiome data analysis. TEM analyzed the cytokines and complement activation products and interpreted

these data. GG, VT, KH, JBH, and SKB interpreted all results of all outcomes in the light of the hypothesis of the study. GG drafted the manuscript with supervision of VT, JBH and SKB. KH, TEM, MD and SKB critically reviewed the manuscript.

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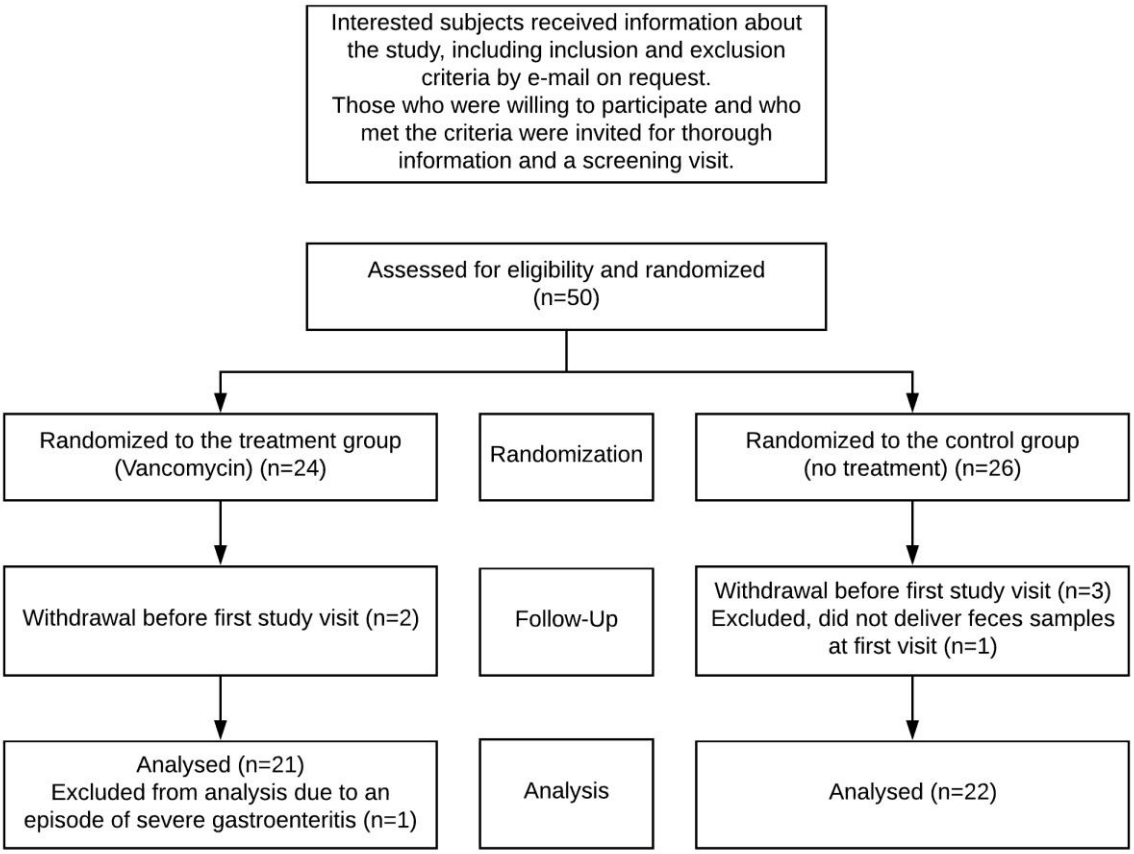
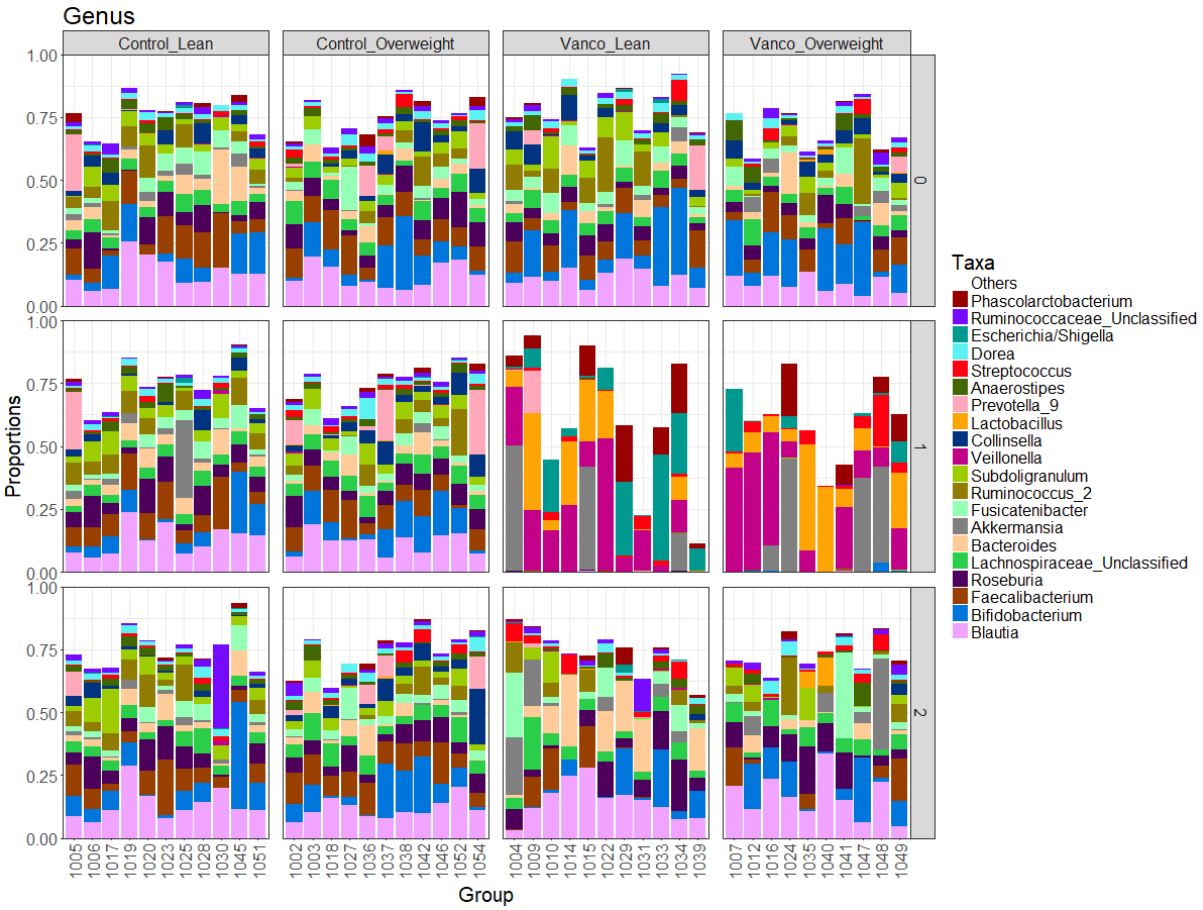


Figure 1. Flow diagram showing recruitment of participants, randomization and completion of the trial.


Genus
Control_Lean
Control_Overweight
Vanco_Lean
Vanco_Overweight
0
1
2
Proportions
1.00
0.75
0.50
0.25
0.00
1005 1006 1017 1019 1020 1023 1025 1028 1030 1045 1051
1002 1003 1018 1027 1036 1037 1038 1042 1046 1052 1054
1004 1009 1010 1014 1015 1022 1029 1031 1033 1034 1039
1007 1012 1016 1024 1035 1040 1041 1047 1048 1049
Group
Taxa
Others
Phascolarctobacterium
Ruminococcaceae_Unclassified
Escherichia/Shigella
Dorea
Streptococcus
Anaerostipes
Prevotella_9
Lactobacillus
Collinsella
Veillonella
Subdoligranulum
Ruminococcus_2
Fusicatenibacter
Akkermansia
Bacteroides
Lachnospiraceae_Unclassified
Roseburia
Faecalibacterium
Bifidobacterium
Blautia

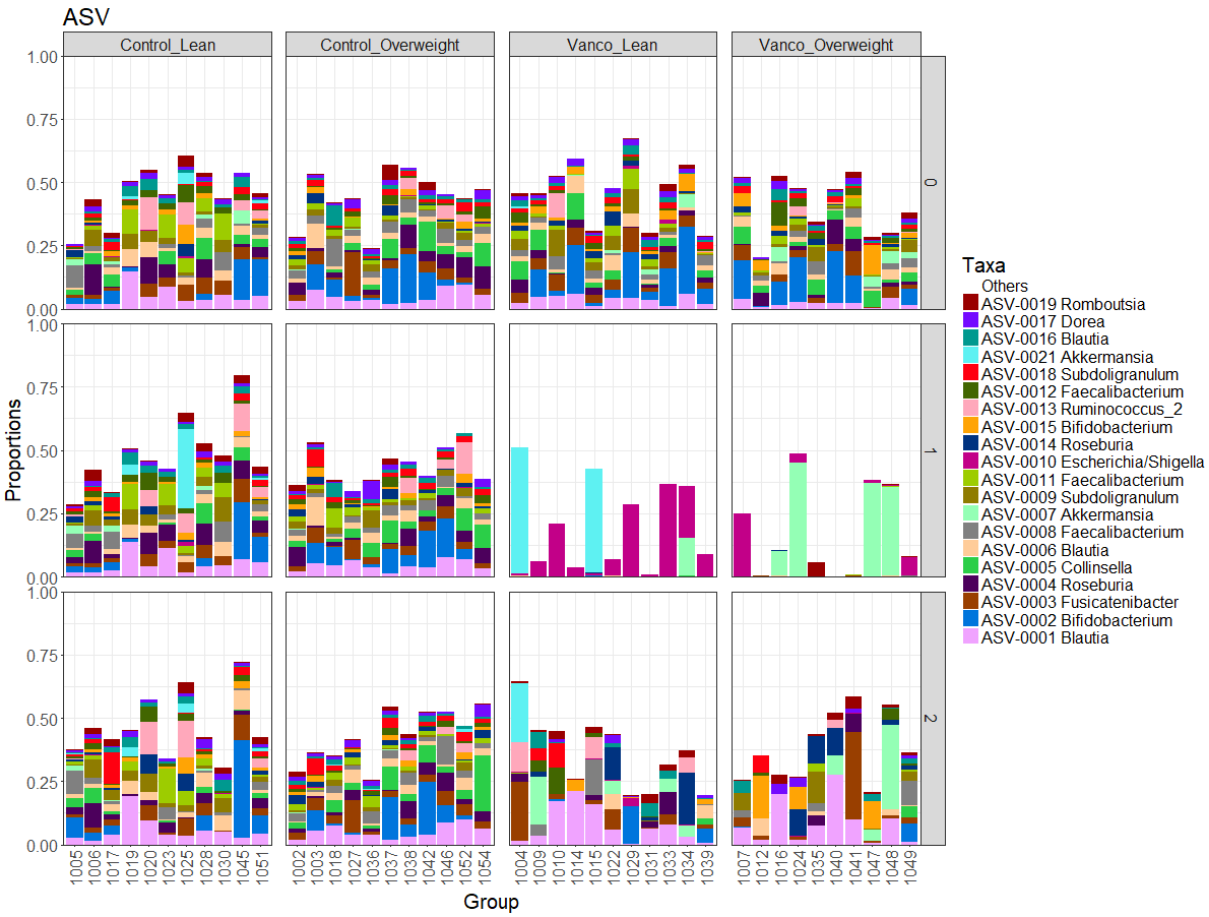


Figure 2. FIGURE text For Genus and ASV , they have to be combined in one Figure .

Gut microbiome composition (amplified sequence variant) in the control and the intervention group at T0 (top), T1 (middle) and T2 (bottom). At T1, the subgroup with BMI <25kg/m^2 (Vanco_Lean) had more bacteria belonging to the *Escherichia/Shigella* group than the subgroup with BMI ≥25kg/m^2 (Vanco_Overweight

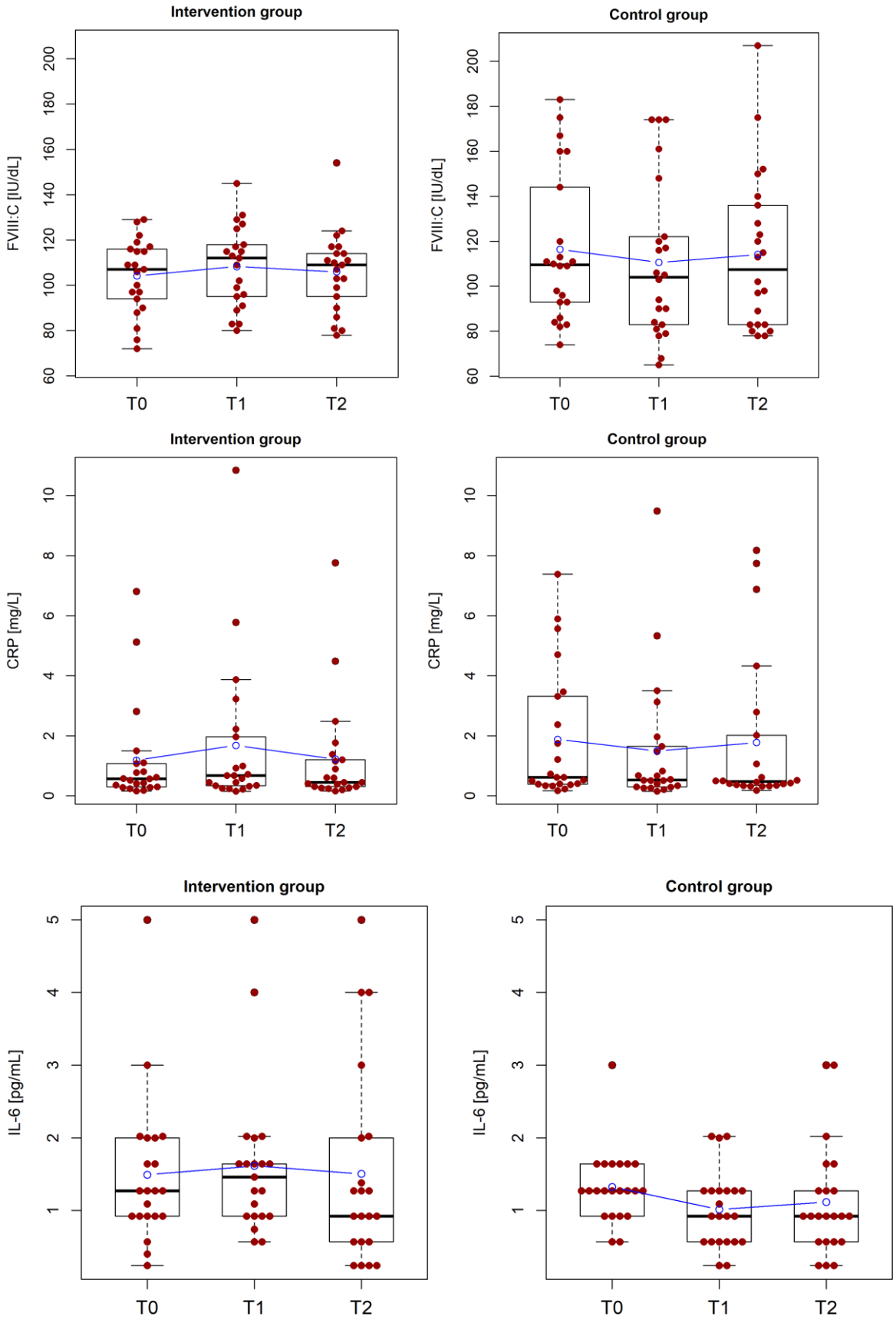


Figure 5. The effect of 7 days intervention with Vancomycin or no intervention on FVIII:C and inflammatory markers

The boxes represent the interquartile range (IQR), with the upper and lower edges (Q_3 and Q_1) of the boxes representing the 75th and the 25th percentiles, respectively. The central horizontal lines within the boxes represent median levels for each group. The upper and lower whiskers follow the default definitions of the boxplot function in R and are defined as min (max(x), Q_3 + 1.5*IQR) and max (min(x), Q_1 - 1.5*IQR), respectively, where x is the data values. The blue dots represent mean values.

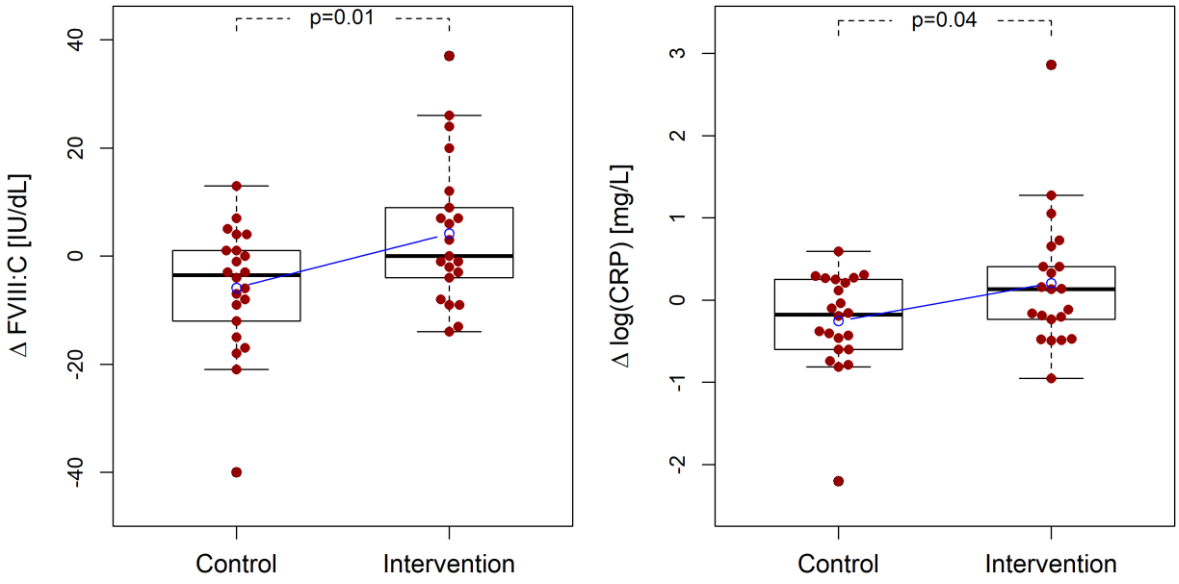


Figure 6. The effect of 7 days intervention with Vancomycin compared to no intervention on change in FVIII:C and hs-CRP

The boxes represent the interquartile range (IQR), with the upper and lower edges (Q_3 and Q_1) of the boxes representing the 75th and the 25th percentiles, respectively. The central horizontal lines within the boxes represent median levels for each group. The upper and lower whiskers follow the default definitions of the boxplot function in R and are defined as min (max(x), Q_3 + 1.5*IQR) and max (min(x), Q_1 - 1.5*IQR), respectively, where x is the data values. The blue dots represent mean values.

Table 1. Baseline characteristics

	Intervention group			Control group		
	All (n=21)	**BMI <25 (n=11)**	**BMI ≥25 (n=10)**	**All (n=22)**	**BMI <25 (n=11)**	**BMI ≥25 (n=11)**
Age (mean, yr)	26.7	24.6	28.9	25.0	24.5	25.5
Female/male (n)	14/7	7/4	7/3	15/7	8/3	7/4
Body mass index (kg/m^2)	25.2	22.8	27.9	26.3	22.3	30.4

BMI: body mass index

Table 2. Effects of 7 days of oral Vancomycin intake versus no intervention on coagulation factor VIII:C and inflammatory parameters

	Intervention group (I) (n=21)			Control group (C) (n=22)			t-test	
Variable	**T0 (mean±SD)**	**T1 (mean±SD)**	**ΔI,T1-T0 (95% CI)**	**T0 (mean±SD)**	**T1 (mean±SD)**	**ΔC,T1-T0 (95% CI)**	**ΔI- ΔC**	**P***
Factor VIII:C (IU/dL)	104±16	108±18	4 (-2 to 10)	116±33	110±35	-6 (-11 to -1)	10	0.01
logCRP†	-0.43±1.0	-0.22±1.1	0.21 (-0.17 to 0.58)	-0.03±1.1	-0.29±1.1	-0.25 (-0.5 to 0.01)	0.46	0.04
Fibrinogen (g/L)	2.6±0.4	2.7±0.4	0.1 (-0.0 to 0.2)	2.8±0.5	2.8±0.5	0.0 (-0.1 to 0.0)	0.1	0.06
IL-6 (pg/mL)	1.5±1.0	1.6±1.1	0.1 (-0.3 to 0.6)	1.3±0.5	1.0±0.5	-0.3 (-0.6 to 0)	0.4	0.10
IL-8 (pg/mL)	1.6±1.7	1.4±1.7	-0.2 (-0.7 to 0.3)	1.4±0.9	0.6± 0.6	-0.8 (-1.2 to -0.3)	0.6	0.10
IL-10 (pg/mL)	2.9±2.2	2.6±3.2	-0.3 (-1.4 to 0.7)	1.6±0.9	0.9±0.8	-0.7 (-1.1 to -0.3)	0.4	0.50
IL-1β (pg/mL)	1.3±0.7	1.2±0.7	-0.1 (-0.3 to 0.1)	1.3±0.6	1.0±0.5	-0.3 (-0.6 to 0)	0.2	0.20
MCP-1 (pg)mL)	5.3±6.0	7.7±8.9	2.4 (-1.2 to 6.1)	3.0±2.3	5.7±5.8	2.7 (0.2 to 5.2)	-0.3	0.92
TNF (pg/mL)	18±14	14±11	-4 (-10 to 2)	16±8	10±9	-6 (-12 to -1)	2	0.50
Neutrophils (x10^9/L)	2.9±1.1	3.1±1.2	0.2 (-0.4 to 0.7)	3.2±1.2	3.3±1.1	0.1 (-0.4 to 0.5)	0.1	0.74
C3bc (CAU/mL)	3.5±2.4	3.6±2.3	0.1 (-1.3 to 1.5)	4.7±2.3	3.6±2.5	-1.1 (-2.1 to -0.1)	1.2	0.15
TCC (CAU/mL)	0.4±0.2	0.4±0.1	0 (0 to 0.1)	0.8±1.6	0.8±1.6	0 (0 to 0)	0	0.36

*t-test for difference in change from T0 to T1 between the intervention and the control group

†High sensitivity C-reactive protein (hs-CRP) was log-transformed to obtain normal distribution