Thrombosis Journal

RESEARCH

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The efficacy of atorvastatin on inflammation and coagulation markers in high-risk thrombotic cancer patients undergoing chemotherapy: a randomized controlled trial

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Abstract

Background Deep vein thrombosis (DVT) is a prevalent complication associated with malignancy. Clinical use of thromboprophylaxis is recommended, however its usage is limited due to bleeding complications, more cost associated, and reluctance to receive anticoagulant injections. Rivaroxaban a relatively easy to administer anticoagulant but it has a risk of bleeding and is expensive. Inflammation is the important factor in pathogenesis of cancer-associated thrombosis. Statins have the anti-inflammatory property that could decrease proinflammatory cytokines. Consequently, statins may be used as thromboprophylaxis for cancer patients receiving chemotherapy.

Objective To provide comparison between atorvastatin and rivaroxaban on affecting inflammatory biomarkers (interleukin 6 [IL-6], C reactive protein [CRP]) and coagulation activation biomarkers (Tissue Factor [TF], prothrombin fragment 1 + 2 [F1 + 2], D-Dimer) in cancer patients at high risk of thrombosis receiving chemotherapy.

Methods A randomized controlled study that was double-blinded and involved high-risk cancer patients undergoing chemotherapy. For up to ninety days, participants were randomized to receiver either atorvastatin 20 mg or rivaroxaban 10 mg daily. The level of plasma of IL-6, CRP, TF, F1 + 2, and D-dimer were assessed 24 h before chemotherapy, 30, 60, and 90 day after chemotherapy. The latest observation carried forward (LOCF) approach was used to examine the data. The laboratory results were evaluated using an independent T test or Mann-Whitney U test prior to and after chemotherapy.

Results Eighty-six randomized patients were enrolled, although both groups showed a decreasing trend in plasma level of IL-6, CRP, TF, F1 + 2, and D-dimer, there were no significant differences between the two groups ($p > 0.05$). In the atorvastatin group, there was a significant correlation between delta level of IL-6 and F1 + 2 ($r = 0.313$, $p = 0.043$) and delta level of CRP and F1 + 2 ($r = 0.398$, $p = 0.009$), whereas in the rivaroxaban group there was a significant correlation between delta CRP and D-dimer level ($r = 0.387$, $p = 0.009$).

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Conclusion Atorvastatin decreases IL-6 and CRP level, which also decreases F1 + 2 level. Atorvastatin did not substantially differ from rivaroxaban in decreasing plasma levels of inflammatory biomarkers IL-6, CRP, and coagulation activation biomarkers TF, F1 + 2, D-dimer in high-risk cancer patients undergoing chemotherapy.

Trial registration ISRCTN71891829, Registration Date: 17/12/2020.

Keywords Inflammation, Coagulation activation, High-risk thrombosis, Cancer patients, Atorvastatin

Introduction

The prevalence of venous thromboembolism (VTE) among individuals diagnosed with cancer is markedly elevated [1–3], particularly in those who received chemotherapy treatment [4, 5]. A significant proportion of VTE occurrences transpires promptly following the commencement of chemotherapy, reaching up to nearly 73% in six months after chemotherapy [6]. Moreover, VTE represents a predominant factor contributing to treatment delays, increased healthcare costs, morbidity, and mortality [7]. The increasing risk of mortality is noted to be three times greater in asymptomatic deep vein thrombosis (DVT) cases [8, 9].

Clinical studies have verified the efficacy and safety of VTE prevention in patients with a variety of medical problems, strengthening the use of evidence-based thromboprophylaxis strategies in daily practice [10–13]. Guidelines have urged for VTE prevention in cancer patients [14–17]. Nonetheless, the use of VTE prevention by healthcare practitioners is still limited [18–21]. The most prevalent reason is cost [18, 20, 22], concerns about bleeding problems [19–21], as well as a lack of insight or confidence in relation to thromboprophylaxis protocols [19], lack of awareness [20, 23] and unwillingness to administer daily injections anticoagulants as prophylaxis [19].

Immunological and inflammatory variables have a key influence in cancer-related VTE [24]. It is still a matter of debate regarding the exact pathomechanism of cancer-related VTE. One theory suggested that cancer and chemotherapy can cause inflammation [25], activating the Nuclear Factor Kappa Beta (NF-κB) signaling cascade and producing pro-inflammatory cytokines [26]. These cytokines such as C-reactive protein (CRP) and Interleukin-6 (IL-6) worsen the procoagulant state predominantly by increasing tissue factor (TF) expression [27]. Tissue factor expression triggers the activation of the coagulation system, leading to elevated levels of circulating thrombin and fibrin formation markers, such as prothrombin fragment 1 + 2 (F1 + 2) and D Dimer [28, 29].

Statins have been shown to inhibit pro-inflammatory cytokines and chemokines; suggesting that they could potentially be used as anti-thrombotic therapy [30]. Statins have significantly decreased the risk of bleeding compared to anticoagulants [31], have cheaper costs, and are commonly available for prescription. Newman et al. examined data from 44 research papers involving 16,495 participants using oral atorvastatin. Severe adverse effects were unusual, and there were no known mortality caused by atorvastatin [32]. However, there is less information on the usage of statins and VTE in cancer patients, and deserved further validation by Randomized Controlled Trial (RCT) research [33].

Rivaroxaban is an oral anticoagulant that, when compared to other parenteral anticoagulant such as unfractionated heparin (UFH) or low molecular weight heparin (LMWH), is easier to administer, namely by single oral administration per day; thus, rivaroxaban shown superior compliance rates [34, 35]. The CASSINI study had demonstrated that Rivaroxaban usage for thromboprophylaxis lower the thrombosis incidence compared to placebo [13], and did not require any monitoring during treatment [36, 37].

To date, there have been no randomized control trial (RCT) studies that compared atorvastatin with rivaroxaban on inflammatory and coagulation activation biomarkers in patients with cancer undergoing chemotherapy with high risk of thrombosis.

We aim to compare the inflammation and coagulation activity between Atorvastatin and Rivaroxaban when given to cancer patients undergoing chemotherapy with high risk of thrombosis by measuring inflammatory and coagulation cascade, specifically IL-6, CRP, TF, F1 + 2, and D-Dimer. This study also aims to use atorvastatin as an alternative thromboprophylaxis for DVT events in cancer patients undergoing chemotherapy.

Methods

Patients

We recruited patients age 18–60 years old with histopathologically confirmed cancer who was chemotherapy-naive and have Khorana risk score of 2 or greater. We asked all patients for their consent, and had them sign the informed consent form for participating in this study.

We excluded patients who had deep vein thrombosis (DVT) at baseline, confirmed by Doppler ultrasonography; had undergone surgery within the previous 14 days; were pregnant; were taking anti-thrombotic medications; had congenital coagulation disorders; had a creatinine clearance of less than 30 mL/min; had aspartate transaminase (AST) levels exceeding three times the upper normal limit; had total bilirubin levels higher than 5 mg/dL;

had creatine kinase (CK) levels more than three times the upper normal limit; had an Eastern Cooperative Oncology Group (ECOG) performance status of 3 or higher; had a history of cardiovascular or cerebrovascular disease, congenital coagulopathy, malignant hypertension, or severe platelet dysfunction; had severe and persistent thrombocytopenia (< 20,000/μL); had ongoing infections; or were experiencing active, major, life-threatening hemorrhages in critical areas (such as intracranial, pericardial, retroperitoneal, intra-articular, intraocular, or intraspinal regions) that could not be controlled by any medical intervention.

The Dr. Kariadi Hospital Institutional Review Board assessed and approved this study protocol, as documented in the Ethical Clearance Statement (665/EC/KEPK-RSDK/2020). The study register number is ISRCTN71891829, with a registration date of 17/12/2020, registry by ISRCTN (International Standard Randomized Controlled Trial Number).

Study design and interventions

This study was a double-blind, randomized controlled trial conducted at Dr. Kariadi Hospital, the primary teaching hospital for the Faculty of Medicine at Diponegoro University. Located in Central Java province, Dr. Kariadi Hospital also serves as a cancer treatment center.

Patients who met the inclusion criteria were enrolled in the study. Before the trial, each patient was informed individually and asked to sign a written informed consent form if they agreed to participate. Relevant patient data, including cancer history, primary tumor site, histopathological profile, and cancer stage, were recorded. Additionally, information such as age, gender, ABO blood type, body mass index (BMI), ECOG performance status, and chemotherapy regimens was carefully documented.

Every participants were put into two random groups. Following the prescription and a study code from the lead researcher, the patient went to the pharmacy department and received a30-day supply of medication without packaging. This study implemented a simple random sampling strategy, which involved generating a collection of random numbers based on sample size. Patients were then selected a random envelope from a closed container and allocated to either control or treatment group using a 1:1 randomization ratio. Twenty milligrams of atorvastatin tablets taken for 24 h was given to treatment group for three months, in addition to chemotherapy regiment. As for the control group, they were supplied with 10 mg of rivaroxaban every 24 h for 3 months, with their respective chemotherapy regiments. The pharmacist employed the third-party randomization, concealing the study medicines and randomly assigning them to participants. The pharmacist subsequently gave the medication to the participants using identical containers containing the respective regiment for each study arms. Sample stratification was not performed in this study. In the event of serious adverse event, investigators were allowed to open the concealment.

Before chemotherapy was administered, venous blood was drawn and stored in a 10 mL EDTA tube. The blood was centrifuged, then blood plasma was separated and stored at minus 80∘C in the laboratory of Dr. Kariadi Hospital Semarang. Laboratory examination includes level of IL-6, CRP, TF, F1 + 2, and D-dimer.

To observe the impaired liver function and myopathy, a physical examination was done on the 7th day. ALT and AST levels were tested to determine the symptoms decreased liver function. Creatine kinase (CK) examination was done if myopathy were found in physical examination. If there was any threefold elevation of AST, ALT, or CK from the upper limit reference value, the study treatment was immediately ceased. If in the 7th day no side effects were observed, further laboratory tests were employed monthly.

The levels of IL-6, CRP, TF, F1 + 2, and D-dimer were measured at the end of the 1st (30th), 2nd (60th day), and 3rd (90th day) month. Laboratory examinations were completed within ± 7 days of the stipulated time. We also checked for hemorrhage. These findings were documented on a previously prepared case research form. After the pre-determined number of study subjects has been met or the research reached has passed its deadline, all data is collected and statistically assessed.

Prediction score

We used the Khorana risk score to discriminate VTE risk among our research participants [38]. Patients were classified into three risk categories: low (scoring 0), intermediate (scores 1–2), and high (score ≥ 3). In this investigation, a Khorana score of 2.14 or above was established as the high-risk category for our patients.

Measurement of research results

The concentration of IL-6, CRP, TF, and F1 + 2 was measured in serum, whereas the amount of D-dimer was determined in citrated plasma. For this measurement, 9.5 mL of venous blood was drawn, 5 mL was put into a vacutainer tube without any anticoagulant, and 4.5 mL was put into a vacutainer tube containing 0.5 mL sodium citrate. The tube without anticoagulant was centrifuged at 2500 g for 15 min, then the serum was separated, coded, and stored at -80°C until testing was carried out.

IL-6 level were examined using enzyme link immunosorbent assay (ELISA) using Elabscience Biotechnology reagents from the USA catalogue number E-EL-H0102 96T. CRP level were measured using ELISA using reagents from Sekisui Medical Co, Ltd, Japan. TF and F1 + 2 level were checked with reagents from Elabscience

Biotechnology USA with catalogue numbers E-EL-H0040 and E-EL-H1793. The results of the ELISA examination were read using an ELISA reader from Biotek, Vermont, USA, at a wavelength of 450 nm. All samples were examined in duplicate. If there are values outside the linearity limits, dilution is carried out and rechecked.

The blood in the citrate tube was also centrifuged at a speed of 2500 g for 15 min. Then the plasma was separated and D-dimer level were examined using immunoturbidimetry using Innovance reagents and an automated blood coagulation analyzer CS-2100i from Sysmex Corporation, Japan.

Statistical analysis

Efficacy of atorvastatin and rivaroxaban on inflammatory and coagulation activation biomarkers was measured by intention-to-treat analysis (ITT) [39]. Thirty-nine data reported missing due to study participants could not complete the study up to 90 days of observation, and the data were analyzed using the last observation carried forward (LOCF) method [40].

Descriptive analysis was employed to provide the study characteristics. The variables were presented in a table to evaluate the equality of mean values and the frequency distribution of variable values across the population. Chi-square test was employed to measure the effect of atorvastatin in comparison with rivaroxaban on DVT incidence.

To assess the effect of atorvastatin and rivaroxaban on level of inflammatory biomarkers and coagulation activation biomarkers on 30th, 60th, and 90th day, a mean difference test was employed (unpaired t-test or Mann-Whitney test). Data was transformed into delta form by subtracting the initial measurement level (baseline data) with monthly serial measurement results. An independent t-test was performed for normally distributed data, while the Mann-Whitney test was used for non-normally distributed data.

The Friedman test was conducted to analyze trends in the reduction of IL-6, CRP, TF, F1 + 2, and D-dimer levels in both the atorvastatin and rivaroxaban groups, Post-hoc Wilcoxon test was carried out to see the trend of decreasing level of IL-6, CRP, TF, F1 + 2, and D-dimer on 30th, 60th, and 90th day in the atorvastatin group and the rivaroxaban group. Spearman's test was carried out to see the correlation between level of inflammatory and coagulation activation biomarkers.

All of the statistical analysis was done using SPSS statistical software version 21 (by IBM, SPSS Inc., USA) We used p value of < 0.05 as our cut-off for statistically-significant test result.

Results

Demographics and characteristics of the study population

A total of 348 who was recently diagnosed cancer patients underwent screening for clinical diagnosis, histopathological data, and the determination of their eligibility for chemotherapy. Among the subjects, 106 individuals satisfied the inclusion criteria; of these, 8 patients (6.7%) presented with deep vein thrombosis (DVT) during the initial screening. Therefore, we included 86 subjects who enrolled and then randomized.

The CONSORT flow diagram was described in Fig. 1.

Table 1 presents the demographics and baseline characteristics of the study population. No significant differences were found in median age or sex between the two groups. Similarly, the atorvastatin and rivaroxaban groups showed no significant variation in terms of blood type, body mass index, ECOG performance status, Khorana score, cancer stage at diagnosis, cancer incidence, chemotherapy regimens, or hemoglobin, leukocyte, and platelet counts.

Among the 86 participants in the study group, 18 individuals (42.8%) terminated the study treatment. In the control group, a similar proportion of 18 subjects (40.9%) also withdrew from the study. A comparative analysis revealed no significant difference in the discontinuation rates between both groups (Odds Ratio [OR], 1.042; 95% confidence interval [CI], 0.674–1.611; $p = 1.000$).

The study treatment was prematurely halted for various reasons, including mortality in 16 (88.9%) and 10 (55.6%) subjects in the atorvastatin and rivaroxaban group, respectively; significant bleeding 4 (22.2%) subjects in control group; primary efficacy endpoint failure in 1 (5.6%) subject of rivaroxaban group; patient-initiated decisions in 1 (5.6%) subject from each group; loss to follow-up in 1 (5.6%) subject from the atorvastatin group; investigator-initiated discontinuation 1 (5.6%) subject from control arm,; and severe corona virus disease-19 (COVID-19) infection in 1 (5.6%) in treatment arm. A statistically significant difference between our groups was identified concerning the reasons for treatment discontinuation ($p = 0.043$). Participants who ceased halted the study were monitored for a period of 90 days to assess bleeding incidence.

One patient in the control group who dropped out of the study passed away before the 90-day observation period ended. In total, eleven patients in the control group died. Most deaths in the treatment group occurred within the first 30 days of surveillance, indicating that the study drug was unlikely to be the cause, as the duration of drug use was short. The primary cause of death in both groups was cancer progression. Additionally, one patient in the atorvastatin group died from a severe COVID-19 infection while in the COVID-19 isolation critical care unit.

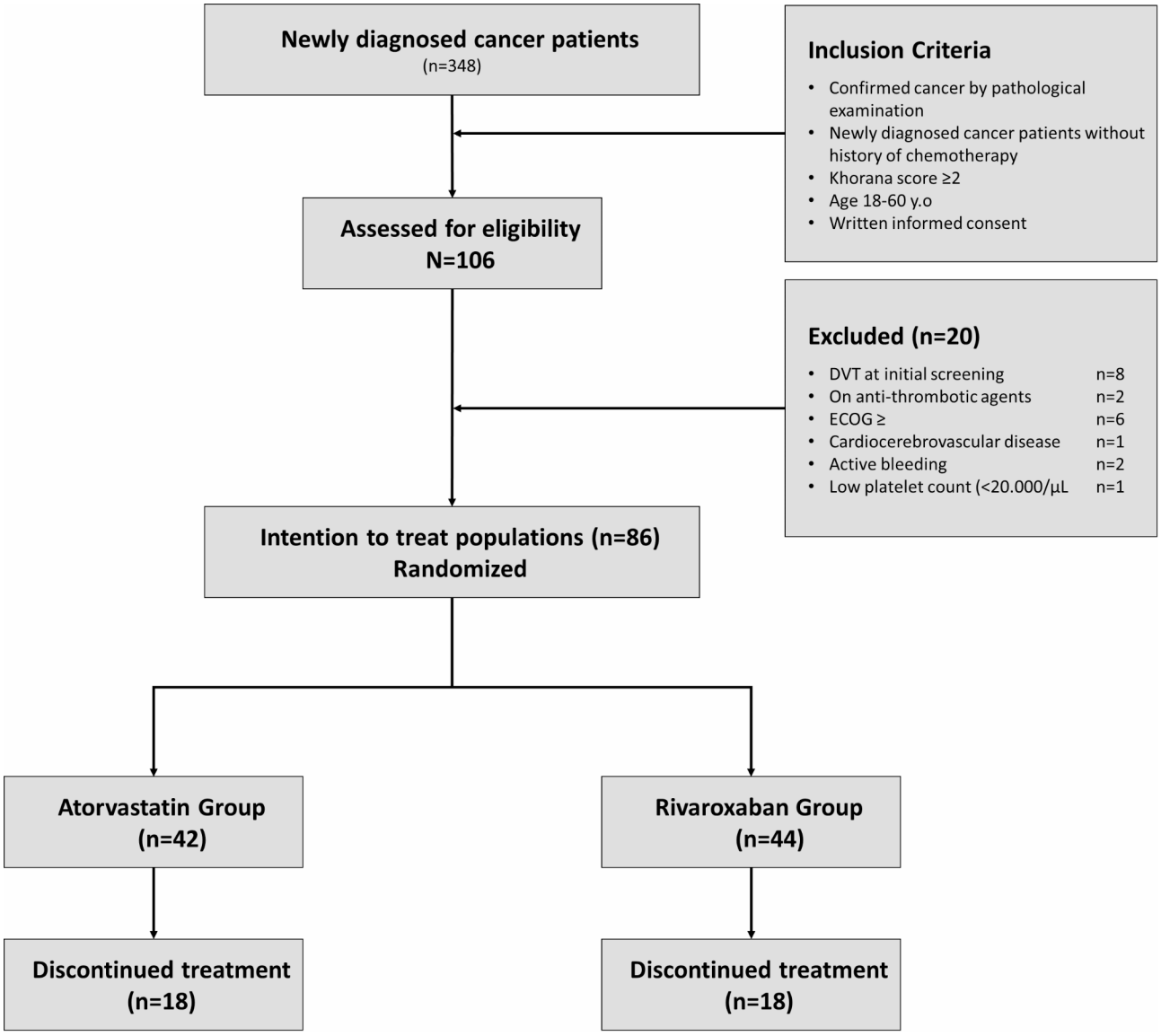


Fig. 1 Study consort

Inflammatory biomarker level and coagulation activity

Table 2 showed baseline value of inflammatory and coagulation activation biomarkers. A significant difference in F1 + 2 level between the atorvastatin group and the pre-treatment rivaroxaban group was observed. ($p = 0.030$)

Comparison of the efficacy of atorvastatin and rivaroxaban on IL-6 level

The Mann-Whitney U Test on the 30th, 60th, and 90th day of atorvastatin group median IL-6 level did not show significant difference compared to rivaroxaban group with $p = 0.816$, $p = 0.360$, and $p = 0.402$ respectively. The median IL-6 level was decreased on day 30 in the atorvastatin group, whereas an increase was observed in the rivaroxaban group on day 30. The atorvastatin group median delta IL-6 levels on day 30 showed no significant compared to rivaroxaban group (Mann-Whitney U Test: Δ 1.5 vs. Δ -0.15, $p = 0.071$).

Elevation of median IL-6 level was showed in both group on day 60 and 90. The median IL-6 level of atorvastatin group on day 90 remained lower compared to median pre-treatment IL-6 level, while in the rivaroxaban group median IL-6 level were higher compared to median pre-treatment IL-6 level. The median delta of IL-6 level of atorvastatin group on day 60 and day 90 revealed no significant difference compared to rivaroxaban groups (Mann-Whitney U Test: Δ 0.30 vs. Δ -0.30, $p = 0.101$; Δ 0.10 vs. Δ 0.00, $p = 0.089$, respectively) (Table 3).

Figure 2 demonstrated a comparison of the efficacy between atorvastatin and rivaroxaban on median IL-6 level. The changes of IL-6 level in both groups were analyzed using the Friedman test. The IL-6 level showed

Table 1 Baseline characteristics of the trial population

Characteristcs	Atorvastatin n = 42	Rivaroxaban n = 44	Total n = 86	p
Age (yr), median (min– max)	43.5 (19–60)	41.5 (20–60)		0.118*
Sex, no. (%)				
Male	21 (50%)	24 (54.5%)	45	0.837*
Female	21 (50%)	20 (45.5%)	41	
Blood type, no. (%)				
O	19 (45.2%)	17 (38.6%)	36	0.688*
Non-O	23 (54.8%)	27 (61.4%)	50	
Body Mass Index (kg/m^2), no. (%)				
Underweight	14 (33.3%)	20 (45.5%)	34	0.107
Normoweight	21 (50%)	22 (50%)	43	
Overweight/obesity	7 (16.7%)	2 (4.5%)	9	
ECOG, no. (%)				
0	26 (61.9%)	29 (52.7%)	55	0.911
1	12 (28.6%)	8 (18.2%)	20	
2	4 (9.5%)	7 (15.9%)	11	
Khorana score, no. (%)				
Intermediate risk (2)	23 (54.8%)	32 (72.7%)	55	0.131*
High risk (≥ 3)	19 (45.2%)	12 (27.3%)	31	
Primary site of cancer, no. (%)				
Very high risk of thrombosis				
Pancreas	5 (11.9%)	2 (4.5%)	7	0.109
Stomach	2 (4.8%)	1 (2.3%)	3	
High risk of thrombosis				
Lung	8 (19%)	7 (15.9%)	15	
Genitourinary	3 (7.1%)	2 (4.5%)	5	
Gynecology	0 (0%)	2 (4.5%)	2	
Lymphoma	4 (9.5%)	5 (11.4%)	9	
Average risk of thrombosis				
Colorectal	14 (33.3%)	17 (38.6%)	31	
Breast	2 (4.8%)	2 (4.5%)	4	
Sarcoma	0 (0%)	2 (4.5%)	2	
Others	4 (9.5%)	4 (9.1%)	8	
Stage of cancer at diagnosis, no. (%)				
I	2 (4.8%)	4 (9.1%)		0.962
II	5 (11.9%)	5 (11.4%)		
III	13 (31%)	11 (25%)		
IV	22 (52.4%)	24 (54.5%)		
Chemotherapy regimen, no. (%)				
5FU *Based*	20 (47.6%)	20 (45.5%)		0.446
Cisplatin *Based*	14 (33.3%)	14 (31.8%)		
R-CHOP	1 (2.4%)	4 (9.1%)		
BEP	1 (2.4%)	1 (2.3%)		
Taxane *Monotherapy*	2 (4.8%)	2 (4.6%)		
Anthracycline *Based*	1 (2.4%)	2 (4.6%)		
ABVD	1 (2.4%)	1 (2.3%)		
GRALL-LYSA	0 (0%)	1 (2.3%)		
De Angelis	0 (0%)	1 (2.3%)		
Laboratory parameters				
Haemoglobin (g/dL)	11.05 (7.7–15.3)	11.25 (7.3–15.9)		0.659
Leukocyte (x10^3/uL)	13 (4.9–37)	11.9 (5.1–21.8)		0.169
Platelet (x10^3/uL)	445 (194–707)	465 (238–951)		0.883

*Chi Square Test; Mann-Whitney U Test

prominent decline in atorvastatin group ($p = 0.024$), however, IL-6 level was shown to increase in the rivaroxaban group, albeit insignificantly ($p = 0.511$).

Comparison of the efficacy of Atorvastatin and Rivaroxaban on CRP level

The median CRP level of atorvastatin group on 30th, 60th, and 90th day showed no significant difference compared to rivaroxaban group with $p = 0.540$, $p = 0.876$, $p = 0.907$, respectively. The decrease in CRP level occurred on day 30 in atorvastatin group, also in rivaroxaban group, however, we did not find significant statistical differences on median delta CRP level between both groups (Δ 0.14 vs. Δ 0.08, $p = 0.526$).

An increase in median CRP level on day 60 was observed both in the atorvastatin and rivaroxaban group. The increase of median CRP level was observed on day 90 in the atorvastatin group, with median CRP level remaining lower than pre-treatment CRP level. Conversely, in the rivaroxaban group there was a decrease, with the median CRP level on the 90th day being lower than pre-treatment. The Mann-Whitney U Test on the median delta of CRP level on the 60th and 90th day showed no significant difference between both groups (Δ 0.00 vs. Δ 0.05, $p = 0.555$, Δ 0.00 vs. Δ 0.02, $p = 0.768$, respectively) (Table 3).

Figure 3 shows a comparison of the efficacy between atorvastatin and rivaroxaban on median CRP level. There was no trend towards a significant decrease in CRP level in the atorvastatin group ($p = 0.070$) as well as in the rivaroxaban group ($p = 0.187$).

Comparison of the efficacy of atorvastatin and rivaroxaban on TF level

Both atorvastatin and rivaroxaban groups median TF level on the 30th, 60th, and 90th day showed no significant difference with $p = 0.323$, $p = 0.306$, and $p = 0.622$ respectively. The median decrease in TF level occurred on day 30 in the atorvastatin group, and increased on day 60 and day 90, with level on day 90 being higher than pre-treatment level. In the rivaroxaban group, there was an increase on the 30th and 60th day, followed by a decrease on the 90th day, with the median TF level on the 90th day being higher than the pre-treatment level.

Table 2 Baseline data on inflammatory and coagulation activity levels

Variable	Atorvastatin *n*=42		Rivaroxaban *n*=44		*p*
	Mean±SD	Median (Min-max)	Mean±SD	Median (Min-max)	
IL-6(pg/mL)	92,06±131,74	43,10 (1,80-506,50)	70,34±111,11	18,20 (0,10-464,5)	0,066†
CRP (mg/dL)	5,20±6,05	3,04 (0,08-27,62)	6,08±7,91	2,71 (0,03-31,11)	0,846†
TF (pg/mL)	76,90±103,83	21,00 (1,40-329,30)	76,59±96,69	31,00 (1,20-360,70	0,935†
F1+2 (pg/mL)	4494.55± 1896.70	5119,00 (585-8993)	3620,52±1780.01	3674,50 (363-6436)	**0,030‡**
D-dimer (ug/L)	3167,62±3827.20	1930,00 (270-19200)	3380.68±3604.99	2255,00 (290-20000)	0,569†

†Mann-Whitney U Test; ‡Independent T Test $p < 0.05$

Prominent decrease of median TF level in atorvastatin group was observed. However, on day 30 the median delta TF level of atorvastatin showed no significant difference compared to rivaroxaban group (Mann Whitney U Test Δ 0.10 vs. Δ 0.00, $p = 0.286$).

Figure 4 depicts a comparison of the efficacy of atorvastatin and rivaroxaban on median TF level. The effect of atorvastatin group in the 60th and 90th day on median delta TF showed no significant difference compared to rivaroxaban group (Δ 0.00 vs. Δ 0.00, $p = 0.781$, Δ 0.00 vs. Δ 0.00, $p = 0.504$, respectively) **(**Table 3**).** There was a trend towards a significant increase in TF level in the atorvastatin group ($p = 0.026$), whereas no marked increase observed in TF level in the rivaroxaban group ($p = 0.782$).

The pre-treatment TF level showed significant difference compared to day 30 TF level from Wilcoxon analysis ($p = 0.004$). However, it wasn't observed on day 60 and 90, with $p = 0.371$ and $p = 0.572$.

Comparison of the efficacy of atorvastatin and rivaroxaban on F1 + 2 level

The median pre-treatment F1 + 2 level in the atorvastatin group was higher than the median F1 + 2 level in the rivaroxaban group with $p = 0.030$. On day 30 and 60, there was no significant difference in median F1 + 2 level between the atorvastatin group and the rivaroxaban group with $p = 0.159$ and $p = 0.108$. On the 90th day, there was a significant difference in the median F1 + 2 level between the atorvastatin group and the rivaroxaban group with $p = 0.049$ with the median F1 + 2 level in the atorvastatin group being higher compared to the rivaroxaban group, as was the case at the start of the study.

Atorvastatin and rivaroxaban group showed median decline in F1 + 2 level occurred on day 30. In fact, the decrease in F1 + 2 level occurred quite a lot in the atorvastatin group, as evidenced by the median of F1 + 2 level in the atorvastatin group, which at the start of the study was quite high and significantly different from the median of F1 + 2 level in the rivaroxaban group, but statistical analysis on day 30 showed no significant difference between the median of F1 + 2 level in the atorvastatin and the rivaroxaban group. The delta decreases in median F1 + 2 level on day 30 of atorvastatin group showed no significant difference compared to rivaroxaban group (Independent T Test: Δ 0.00 vs. Δ 2.00, $p = 0.459$).

Both atorvastatin and rivaroxaban group showed increase in the median F1 + 2 level with lower number than the initial level on day 60 and 90. The median delta of F1 + 2 level on day 60 and 90 of atorvastatin group showed no significant difference compared to rivaroxaban group (Mann-Whitney U Test: Δ 0.00 vs. Δ 0.00, $p = 0.729$; Δ 0.00 vs. Δ 7.50, $p = 0.890$, respectively) **(**Table 3**).**

Figure 5 shows a comparison of the effect of atorvastatin compared to rivaroxaban at median F1 + 2 level. Changes of F1 + 2 level between both group were analyzed using the Friedman test. There was no significant tendency to decrease F1 + 2 level in the atorvastatin group ($p = 0.356$) as well as no significant tendency to decrease F1 + 2 level in the rivaroxaban group ($p = 0.765$).

Comparison of the efficacy of Atorvastatin and Rivaroxaban on D-dimer level

The difference of median D-dimer level of atorvastatin group on the 30th, 60th, and 90th day compared to rivaroxaban group wasn't significant with $p = 0.863$, $p = 0.866$, and $p = 0.945$ respectively. A median decrease in D-dimer level occurred on day 30 in both groups, but the difference significancy wasn't observed. (Δ 260 vs. Δ 135, $p = 0.764$).

The median D-dimer level decline was observed in both atorvastatin and rivaroxaban group on the 60th day, then there was an increase on the 90th day with the median D-dimer level remaining lower than the median pre-treatment D-dimer level. The median delta D-dimer level

Table 3 Comparison of the efficacy of atorvastatin and rivaroxaban on IL-6, CRP, TF, F1 + 2 and D-dimer levels

	Atorvastatin (n = 42)		**Rivaroxaban (n = 44)**		**p**
	***Mean* ± SD**	***Median* (*Min-max*)**	***Mean* ± SD**	***Median* (*Min-max*)**	
IL-6 Levels (pg/mL)					
Pretreatment	92,06 ± 131,74	43,10 (1,80–506,50)	70,34 ± 111,11	18,20 (0,10–464,50)	0,066†
Day 30	85,87 ± 144,57	25,60 (1,80–506,50)	77,35 ± 121,86	21,95 (0,40–491,50)	0,816†
Delta 1	6,19 ± 78,83	1,50 (-396,20–196,80)	-7,01 ± 85,68	-0,15 (-288,40–213,60)	0,071†
Day 60	103,68 ± 165,00	28,00 (2,70–506,50)	79,32 ± 122,58	22,65 (0,60–491,50)	0,360†
Delta 2	-11,62 ± 94,52	0,30 (-396,20–100,70)	-8,97 ± 88,87	-0,30 (-288,40–282,40)	0,101†
Day 90	102,82 ± 165,17	28,60 (2,30–506,50)	81,17 ± 121,89	24,15 (0,40–491,50)	0,402†
Delta 3	-10,76 ± 118,13	0,10 (-419,20–247,10)	-10,83 ± 89,45	0,00 (-288,40–285,00)	0,089†
CRP Levels (mg/dL)					
Pretreatment	5,20 ± 6,05	3,04 (0,08–27,62)	6,08 ± 7,91	2,71 (0,03–31,11)	0,846†
Day 30	4,06 ± 5,31	1,31 (0,02–19,07)	5,73 ± 7,83	1,52 (0,01–31,11)	0,540†
Delta 1	1,14 ± 5,34	0,14 (-11,07–27,35)	0,35 ± 4,65	0,08 (-19,35 – 13,37)	0,526†
Day 60	5,75 ± 7,06	2,49 (0,02–27,09)	6,50 ± 8,34	2,19 (0,04–31,11)	0,876†
Delta 2	-0,56 ± 7,41	0,00 (-21,52 – 27,52)	-0,42 ± 5,92	0,05 (-20,94 – 13,37)	0,555†
Day 90	5,96 ± 8,32	2,75 (0,04–40,06)	6,61 ± 8,33	1,77 (0,02–31,11)	0,907†
Delta 3	-0,76 ± 8,80	0,00 (-39,00–27,08)	-0,54 ± 5,93	0,02 (-20,94 – 13,37)	0,768†
TF Levels (pg/ml)					
Pretreatment	76,90 ± 103,83	21,00 (1,40–329,30)	76,61 ± 96,66	31,00 (1,20–360,70)	0,935†
Day 30	54,36 ± 80,12	16,65 (1,20–329,30)	67,74 ± 88,30	37,85 (1,30–342,90)	0,323†
Delta 1	22,54 ± 56,98	0,10 (-34,40–243,40)	8,85 ± 45,77	0,00 (-142,60–185,70)	0,286†
Day 60	59,33 ± 79,78	25,65 (1,5-329,30)	74,48 ± 90,50	47,50 (0,60–365,60)	0,306†
Delta 2	17,57 ± 73,13	0,00 (-103,40–265,40)	2,11 ± 67,72	0,00 (-273,70–249,10)	0,781†
Day 90	66,53 ± 79,30	33,55 (1,30–329,30)	78,80 ± 95,85	37,70 (1,50–365,60)	0,622†
Delta 3	10,37 ± 64,17	0,00 (-97,80–203,30)	-2,21 ± 71,87	0,00 (-273,70–204,30)	0,504†
F1 + 2 Levels (pg/mL)					
Pretreatment	4494,55 ± 1896,70	5119,00 (585–8993)	3620,52 ± 1780,01	3674,50 (363–6436)	**0,030‡**
Day 30	3814,40 ± 2044,99	3955,00 (193–8993)	3215,66 ± 1860,38	3022,00 (237–8445)	0,159‡
Delta 1	680,14 ± 1469,40	0,00 (-2418-4328)	404,86 ± 1923,82	2,00 (-4779-4994)	0,459‡
Day 60	4092,19 ± 1773,52	4062,50 (69-8993)	3462,18 ± 1822,03	3233,50 (353–8445)	0,108‡

Table 3 (continued)

	Atorvastatin (n = 42)		Rivaroxaban (n = 44)		p
	Mean ± SD	*Median* (*Min-max*)	*Mean* ± SD	*Median* (*Min-max*)	
Delta 2	402,35 ± 1394,04	0,00 (-2551-3790)	158,34 ± 1694,50	0,00 (-4779-4084)	0,729†
Day 90	4273,95 ± 1604,51	4205,00 (417–8993)	3529,00 ± 1835,69	3570,50 (82-8445)	**0,049‡**
Delta 3	220,60 ± 1380,76	0,00 (-3072-4012)	91,52 ± 1894,42	7,50 (-4779-3449)	0,890†
D-Dimer Levels(ug/L)					
Pretreatment	3167,62 ± 3827,20	1930,00 (270-19200)	3380,68 ± 3604,99	2255,00 (290-20000)	0,569†
Day 30	2277,76 ± 3252,08	1450,00 (270-19200)	2878,64 ± 4165,22	1370,00 (270-20000)	0,863†
Delta 1	889,86 ± 2289,68	260,00 (-1910-12730)	502,05 ± 2881,72	135,00 (-9040-9730)	0,764†
Day 60	2544,29 ± 3396,72	1330,00 (270-19200)	2845,36 ± 3564,05	1345,00 (270-15860)	0,866†
Delta 2	623,33 ± 2342,45	150,00 (-5070-11010)	535,32 ± 3212,83	220,00 (-9040-9910)	0,934†
Day 90	2475,45 ± 3283,09	1685,00 (190-19200)	3030,59 ± 4190,23	1428,00 (270-20000)	0,945†
Delta 3	692,17 ± 2526,04	125,00 (-4590-13320)	350,09 ± 4570,87	145,00 (-18820-12730)	0,825†

†Mann-Whitney U Test, ‡Independent T Test p < 0.05

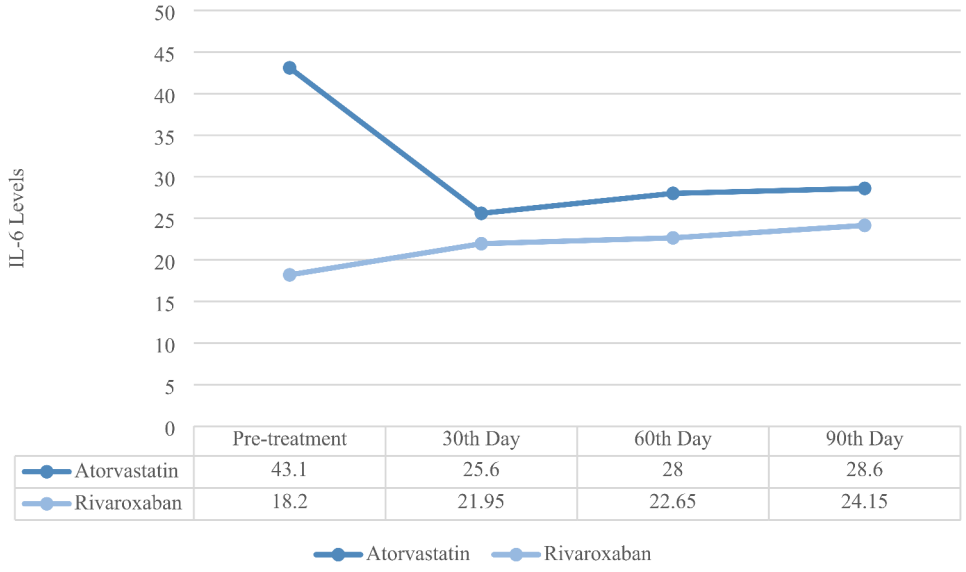


Fig. 2 Comparison of the efficacy of atorvastatin and rivaroxaban on median value of IL-6 levels. †Mann-Whitney U Test, * Friedman Test

of atorvastatin group on day 60 and 90 showed no significant difference compared to rivaroxaban group (Mann-Whitney U Test: Δ 150 vs. Δ 200, $p = 0.934$; Δ 125 vs. Δ 145, $p = 0.825$, respectively) (Table 3). Figure 6 shows a comparison of the efficacy between atorvastatin and rivaroxaban group on median D-dimer level. There was a trend towards D-dimer level decline in both atorvastatin group ($p = 0.001$) and rivaroxaban group ($p = 0.013$).

Relationship between inflammatory biomarkers and coagulation activation

A correlation analysis was carried out between inflammatory biomarkers and coagulation activation on the 90th day delta using the Spearman's test. From the analysis, it showed significant relationship between delta level of IL-6 and F1 + 2 ($r = 0.313$, $p = 0.043$) and delta level of CRP and F1 + 2 ($r = 0.398$, $p = 0.009$) in the atorvastatin

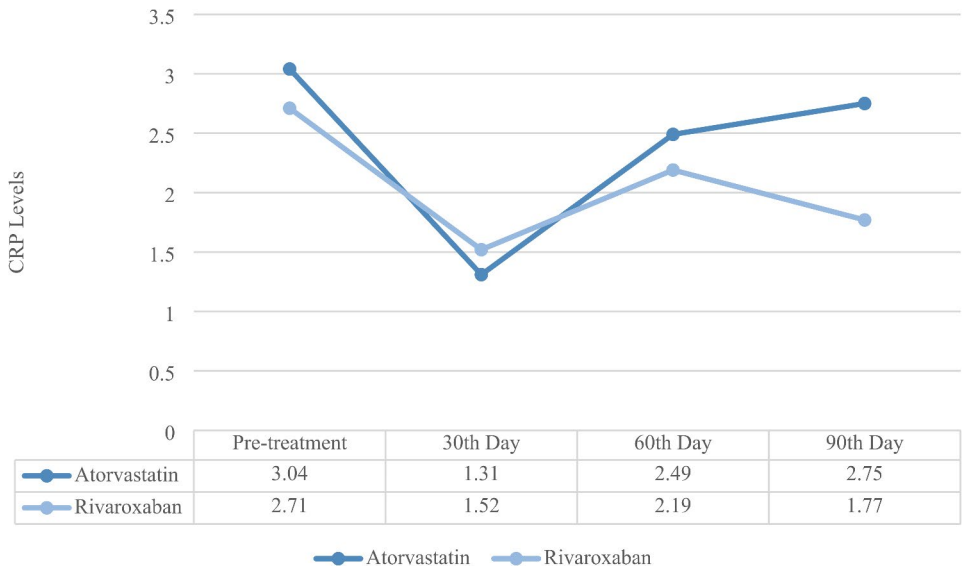


Fig. 3 Comparison of the efficacy of atorvastatin and rivaroxaban on median value of CRP levels. †Mann-Whitney U Test, * Friedman Test

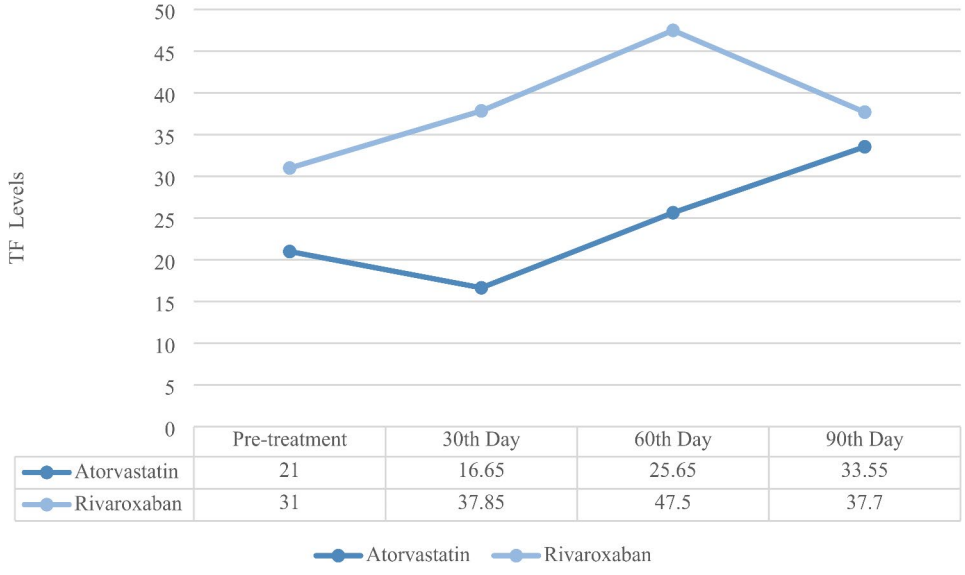


Fig. 4 Comparison of the efficacy of atorvastatin and rivaroxaban on median value of TF levels. †Mann-Whitney U Test, * Friedman Test, Wilcoxon Test

group, whereas significant relationship was observed between delta CRP and D-dimer level in the rivaroxaban group ($r = 0.387$, $p = 0.009$) (Table 4).

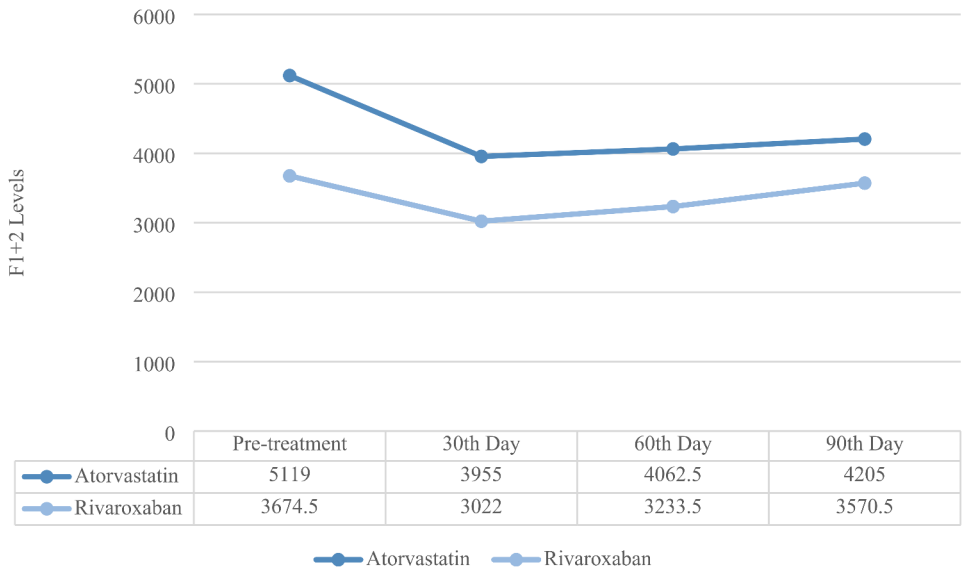
Primary efficacy end point

The effect of atorvastatin administration was observed using intention-to-treat analysis over a 90-day observation period, regardless of whether the incidence of deep vein thrombosis occurred after discontinuation of the study drug. All study subjects who had discontinued the study drug before the end of the study underwent a Doppler ultrasound examination on the 90th day. In this study, there was 1 (2.3%) and 1 (2.2%) DVT case in the atorvastatin and rivaroxaban group, respectively (OR 0.953; 95% CI, 0.240–3.971; $p = 1{,}000$) [41].

Primary safety end point

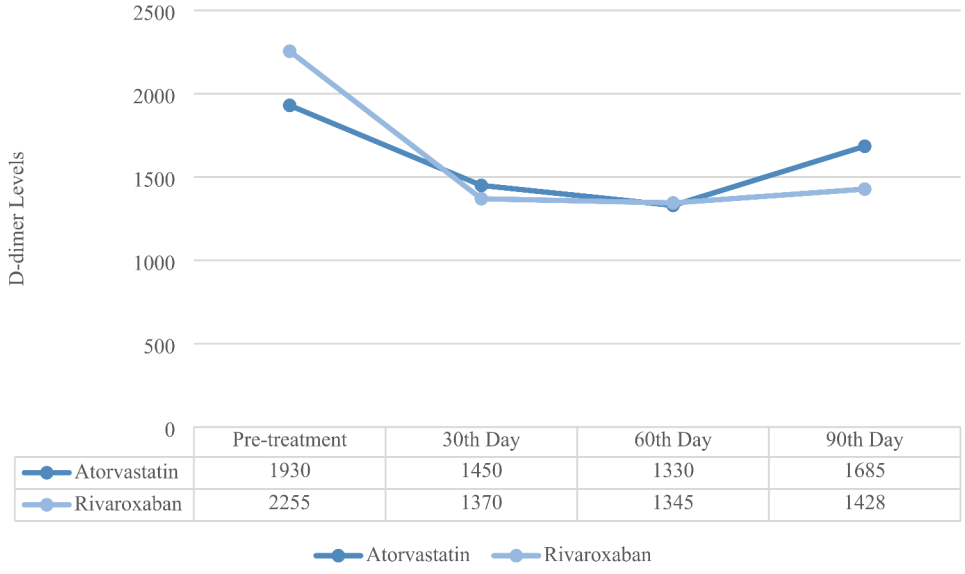
In this study, the primary safety endpoint during the 90 day observation period occurred in 2 (4.8%) and 12 (27.3%) subjects in the atorvastatin and the rivaroxaban group, respectively. There was a significant difference in terms of major bleeding incidence between the atorvastatin group and the rivaroxaban group (OR 0.257; 95% CI, 0.07–0.94; $p = 0.007$) [41].

Liver function impairment and signs of myopathy that progressed to rhabdomyolysis wasn't observed after



	Pre-treatment	30th Day	60th Day	90th Day
Atorvastatin	5119	3955	4062.5	4205
Rivaroxaban	3674.5	3022	3233.5	3570.5

Fig. 5 Comparison of the efficacy of atorvastatin and rivaroxaban on median value of F1 + 2 levels. ‡Independent T Test, * Friedman Test



	Pre-treatment	30th Day	60th Day	90th Day
Atorvastatin	1930	1450	1330	1685
Rivaroxaban	2255	1370	1345	1428

Fig. 6 Comparison of the efficacy of atorvastatin and rivaroxaban on median value of D-dimer levels. †Mann-Whitney U Test, * Friedman Test

evaluation of 90-day observation period as previously shown by our group [41].

Discussion

The purpose of this study is to investigate the efficacy of atorvastatin in the modulation of inflammatory and coagulation biomarkers in high-risk thrombosis chemotherapy patients due to cancer. The results of our analysis revealed the potential significance of atorvastatin in addressing thrombotic risk within this cohort, although it is evident that further research is required. Significant reduction in inflammatory biomarkers was observed, particularly IL-6 and CRP, after atorvastatin treatment. This reduction in pro-inflammatory cytokines correspondingly led to diminished coagulation activation, as evidenced by lower concentrations of thrombin formation indicators such as TF, F1 + 2, and D-dimer. This results consistent with previous studies showing the anti-inflammatory characteristics of statins, which may contribute to their thromboprotective effects [30]. The correlation between the decreases in IL-6, CRP, and F1 + 2 level further showed that atorvastatin's anti-inflammatory effect may reduce thrombin generation and lower the risk of VTE [27–30].

Comparative analysis shows that by day 30, atorvastatin administration led to a decrease in IL-6, CRP, TF, F1 + 2, and D-dimer level. Contrary, rivaroxaban increased IL-6 and TF level but decreased CRP, F1 + 2, and D-dimer

Table 4 Relationship between Delta levels of inflammatory biomarkers and coagulation biomarkers day 90

	Inflammatory biomarkers	Coagulation activation biomarkers	*r*	*p*
Atorvastatin	IL-6	TF	0.210	0.181*
		F1+2	0.313	0.043*
		D-dimer	0.259	0.098*
	CRP	TF	-0.19	0.907*
		F1+2	0.398	**0.009***
		D-dimer	0.178	0.258*
Rivaroxaban	IL-6	TF	0.191	0.213*
		F1+2	0.094	0.543*
		D-dimer	0.171	0.267*
	CRP	TF	-0.117	0.448*
		F1+2	-0.214	0.162*
		D-dimer	0.387	**0.009***

* Spearman's Test $p < 0.05$

level. After day 30, The atorvastatin group has slight increase in the level of IL-6, F1 + 2, and D-dimer after day 30. However, the increase remains lower than pre-treatment level by day 90. This indicated that atorvastatin effectively reduced these biomarkers for up to 90 days.

TF level in this study increased compared to baseline level in both group. This phenomenon can be elaborated with several explanations. Firstly, the coagulation assays currently employed in clinical settings are not optimized for the quantification of endogenous TF level. Secondly, TF level were assessed via the enzyme-linked immunosorbent assay (ELISA) methodology, which is associated with limitations in sensitivity and specificity, and its clinical relevance remains limited to analyzing patient samples. Third, other methods more reliable than ELISA can be employed, such as flow cytometry, as flow cytometry enables the detection of TF expression on various cell types through the use of fluorescently labelled antibodies targeting TF [42]. Lastly, as previously reported by Parhami-Seren B et al., regarding TF's association to hemostasis and certain disease still uncertain because numerous contradictory studies about its existence and concentration in the blood [43].

A notable reduction in inflammatory biomarkers and coagulation activation was observed up to day 30 in the atorvastatin group. Atorvastatin effectively suppressed inflammation, leading to a decrease in IL-6 and CRP concentrations among high-risk thrombotic cancer patient. This reduction in pro-inflammatory cytokines inhibited TF expression and reduced coagulation activation, as shown by decreased level of thrombin formation markers like F1 + 2 and D-dimer. On day 60 and 90, atorvastatin continued to exhibit lower level of IL-6, CRP, F1 + 2, and D-dimer, albeit to a lesser extent than observed on day 30. Nevertheless, the efficacy in diminishing TF level was found to be significant only up to day 30, likely attributable to the limitations in sensitivity and specificity of the ELISA assay utilized for quantifying TF level.

The significant decrease in the levels of inflammatory biomarkers and coagulation activation observed on the 30th day could be attributed to the fact that cancer patients are typically in a state of high inflammation prior to chemotherapy. Chemotherapy itself exacerbates inflammation; so, when combined with 30 days of atorvastatin therapy, there is a notable reduction in inflammatory markers. Meanwhile, by the 60th and 90th days, the inflammation caused by cancer tends to diminish as the cancer cells becomes better controlled due to the effects of chemotherapy drugs. In this study, atorvastatin was still effective in reducing IL-6, CRP, F1 + 2, and D-dimer levels on the 60th and 90th days, but the reduction was not as pronounced as that observed on the 30th day.

To elaborate on how atorvastatin reduced inflammatory and coagulation activation biomarkers, we conducted a correlation analysis between these markers on the 90th day delta using Spearman's test. The findings indicated a statistically significant correlation within the atorvastatin cohort between variations in IL-6 and F1 + 2 level, as well as between alterations in CRP and F1 + 2 level. This suggests that atorvastatin effectively inhibited IL-6 and CRP, thereby resulting in a decline in F1 + 2 level, subsequently contributing to the reduction of DVT occurrences in our study.

Park OY et al., reported 2 months of therapy with simvastatin compared with the placebo group on IL-6 level in normocholesterolemic acute coronary syndrome patients. Simvastatin significantly lowered IL-6 level compared with the placebo group [44]. This study also showed that atorvastatin could reduce IL-6 level. Therefore, it showed the risk reduction potential of statin in venous thromboembolism (VTE) events in healthy people by lowering inflammatory biomarkers Research by Adam NB et al., showed that patients who used statins had lower level of C-reactive protein than patients who did not use statins [45]. Research by Park OY et al., also suggests that simvastatin therapy significantly reduced hsCRP level compared with the placebo group [44].

Our study shows a reduction in CRP level within both the atorvastatin and rivaroxaban group, albeit with no statistically significant difference in the reduction of CRP level between the atorvastatin and rivaroxaban group. This finding suggests that atorvastatin and rivaroxaban exhibit comparable efficacy in suppressing the inflammatory biomarker CRP.

The TF expression regulatory mechanism still unclear, even though it plays a role on formation of thrombus during ACS (acute coronary syndrome). Statins demonstrate efficacy in individuals diagnosed with ACS. Research conducted by Eto M et al. indicates that the induction of TF within the endothelium is modulated by

Rho/Rho-kinase, Akt, and p38 MAP kinase pathways. Simvastatin mitigates TF induction via the inhibition of Rho/Rho-kinase and the activation of Akt. The findings of this study offer novel perspectives on the implications of statins in the context of acute coronary syndromes [46].

Ay C et al. previously reported that F 1 + 2 level serve as independent predictors for the occurrence of venous thromboembolism (VTE) in cancer patient [47]. Prior research had also demonstrated that simvastatin diminishes in vivo clotting activation along with monocyte TF expression. A study conducted by Ferro et al. aimed to evaluate whether simvastatin exerts a direct impact on clotting activation through an in vitro methodology in which the clotting system is activated by monocytes stimulated with lipopolysaccharide (LPS). Simvastatin can disrupt the expression of monocyte TF directly, thus the production of thrombin is suppressed [48].

This study also showed that there was a decrease in TF level but only up to the 30th day of the study and a decrease in F1 + 2 level in both the atorvastatin group and the rivaroxaban group. No statistically significant differences were noted in delta TF and F1 + 2 level when comparing the atorvastatin and rivaroxaban group. This implied that atorvastatin and rivaroxaban exhibit comparable efficacy in the suppression of coagulation activation biomarkers, namely TF and F1 + 2.

Research by Schorling RM et al. shows a saline D dimer level, but not IPF, MPV, or P-selectin were associated with the risk of developing VTE in Ambulatory Cancer Patients (HR 6.9; $p = 0.021$) [49]. Schol-Gelok S et al., previously studied the effect of statins on D-dimer level in patients with suspected pulmonary embolism, revealed that statins was associated with a decrease in D-dimer level [50]. Research by Adam NB et al., also shows that patients who used statins had D-dimer level (– 9%) lower than patients who did not use statins ($p < 0.05$) [45].

The decline of D-dimer level in atorvastatin and rivaroxaban group are consistent with our study. However, the difference are not significant in both group. This suggests that atorvastatin and rivaroxaban have the same efficacy in suppressing the coagulation activation biomarker D-dimer.

The findings of the studies above show that the delta reduction of atorvastatin group in the inflammatory biomarkers IL-6, CRP, and the coagulation activation biomarkers TF, F1 + 2, and D-dimer were not significantly different compared to rivaroxaban group, which is a standard anticoagulant drug for DVT prophylaxis in high-risk thrombosis cancer patients. The results of the study reported by Setiawan B, et al., with the same research subjects, showed that atorvastatin had similar efficacy compared to rivaroxaban in lowering DVT incidence, minimizing bleeding risk, and cost-effectiveness as thromboprophylaxis for chemotherapy patients with high-risk of thrombosis [41].

Limitations

There were several limitations of our study. First, we did not measure NF-κB expression. The translocation process of the NF-κB complex occurs in the cell nucleus [51], necessitating accurate quantitative assessment of NF-κB translocation through the application of immunofluorescence microscopy. Examinations carried out on blood plasma will result in inaccurate quantitative NF-κB examination results [52].

Second, TF level was examined using the ELISA method. The ELISA technique for quantifying TF level is associated with challenges related to sensitivity and specificity, which consequently limits its clinical applicability to patient sample analyses. In contrast, flow cytometry could provide enhanced sensitivity and specificity for the measurement of TF expression [42].

It is still a matter of debate whether the intrinsic pathway or the extrinsic pathway that play major role of VTE in cancer patients. This study focused on TF as one of the extrinsic pathway biomarkers. We suggested further study to include biomarkers of intrinsic pathway to better understand the pathophysiology of VTE in cancer patients.

Chemotherapy outcome result was not available in this study, as our study only focused on 90-day of treatment, whereas most chemotherapy regiments were not completed in this time range. A further study in longer duration and evaluating the chemotherapy response is encouraged.

Nearly 42% of the randomized patients withdrew prior to completion of the 90-day observation period for various reasons. This is due to most of the patients are in an advanced stage and are unexpected due to this stage of disease, as previously reported in studies of DVT prophylaxis to cancer patients such as the CASSINI [13], PROTECHT [53], and SAVE-ONCO studies [54].

Lastly, our study did not have negative control group, thus an ideal comparison could not be observed. However, ethical consideration was our main reason not including a placebo group, as our main inclusion criteria was cancer patients with high risk of thrombosis. Future studies with different approach were suggested to better study atorvastatin effects on inflammatory biomarker without having to compromise ethical issues.

Conclusion

Daily 20 mg Atorvastatin for 3 months can reduce both biomarkers of inflammatory (IL 6 & CRP) and coagulation activation (TF, F1 + 2, D-dimer) in chemotherapy patient with high risk of thrombosis in contrast with 10 mg Rivaroxaban.

Administration of atorvastatin was effective in reducing inflammatory biomarkers and coagulation activation biomarkers until the 30th day of the study, so it is necessary to perform a larger and multicenter RCT study involving more patients to evaluate the efficacy of atorvastatin in reducing inflammatory biomarkers and coagulation activation biomarkers, which are expected to prevent DVT in chemotherapy patients with high risk of thrombosis. This aligns with the objective of incorporating atorvastatin as a potential component of DVT prophylaxis regimens for chemotherapy patients.

The impact of atorvastatin on quantitative NF-κB expression need to be studied using immunofluorescence microscopy that can quantitatively detect NF-κB translocation in the cell nucleus as central mediator of development and progression of inflammation that led to DVT. Further research needs to be carried out by analyzing TF level using flow cytometry examination, which has better sensitivity and specificity in measuring TF level.

Abbreviations

ACS	Acute Coronary Syndrome
AST	Aspartate Transaminase
BMI	Body mass index
CK	Creatine Kinase
COVID-19	Corona virus disease-19
CRP	C-reactive Protein
DVT	Deep Vein Thrombosis
ECOG	Eastern Cooperative Oncology Group
ELISA	Enzyme link immunosorbent assay
F1 + 2	Prothrombin Fragment 1 + 2
IL-6	Interleukin-6
ISRCTN	International Standard Randomized Controlled Trial Number
ITTL	intention-to-treat
LMWH	Low Molecular Weight Heparin
LOCF	Latest Observation Carried Forward
LPS	lipopolysaccharide
NF-κB	Nuclear Factor Kappa Beta
RCT	Randomized Controlled Trial
TF	Tissue Factor
UFH	Unfractioned heparin
VTE	Venous Thromboembolism

Acknowledgements
We would like to thank Mika Lumbantobing, M.D., and Suyono, M.D. from the Department of Internal Medicine, Dr. Kariadi Hospital, Diponegoro University, Semarang, Indonesia for their assitance in cancer patient and to all of dr. Kariadi Hospital colleagues who supported us in patient recruitment. We also express gratitude for support from Gunawan Santosa M.D., Ph.D. for his radiological expertise in performing duplex ultrasonography of the extremities, and Suhartono, M.D., Ph.D. from the Clinical Epidemiology Unit of Diponegoro University for the statistical support. Last but not least, the authors would like to thank all of the participants and their family for contributing by enrolling in the study.

Author contributions
B.S. in concepting and designing the study, data review, writing initial manuscript, analyzed the data and reviewed the final manuscript. W.B., T.S.W. recruited study participants, collected the samples and data, and reviewed the manuscript. D.R. assisted with study oversight, collected the data, analyzed and carried out the statistical analysis, and reviewed the manuscript. E.A. P., D.S. supervised the study, critically appraised the manuscript. A.W.S., T.I.W., I.R. supervised the study, data review and analysis, critically appraised the manuscript. R.D.S., C.S. in concepting and designing the study, data review and analysis, reviewed the final manuscript, study supervision, critically appraised the manuscript.All authors were present in interpretation and hearing of the results, then contributed, read and approved the final manuscript hereby submitted for publication.

Funding
Not applicable.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This single-centre observational study was approved by Ethical Committee of Dr. Kariadi Hospital.

Consent for publication
All authors have consented for the publication.

Competing interests
The authors declare no competing interests.

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Received: 15 October 2024 / Accepted: 26 February 2025
Published online: 19 March 2025

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