



Comparing effects of different routes of heparin administration on the serum biomarkers of thrombosis

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Background: Association between thrombosis pathogenesis and inflammatory conditions has been reported. Also inflammatory biomarkers have been proposed for prediction of thrombosis events. **Objectives:** Effects of different methods of heparin administration (subcutaneous vs continuous infusion) as thromboprophylaxis on the biomarkers of thrombosis have been evaluated. **Methods:** Serum levels of hsCRP, IL-10 and P-selectin as the biomarkers of thrombosis were measured at baseline, days 3 and 7 during the patients' hospitalization period. **Results:** Changes in the serum levels of thrombosis biomarkers (hsCRP, IL-10 and P-selectin) were comparable between the subcutaneous and continuous infusion groups. **Conclusion:** Both subcutaneous injection and continuous infusion of heparin as thromboprophylaxis showed same effects on the thrombosis biomarkers.

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Critically ill patients are more vulnerable to thrombosis due to immobility and concomitant inflammatory conditions. Deep-vein thrombosis (DVT) and pulmonary embolism (PE), the two subtypes of venous thromboembolism (VTE), are associated with high rate of morbidity and mortality. Thromboprophylaxis is highly recommended for prevention of VTE events in this population [1–5].

Low-dose unfractionated heparin (UFH), low-molecular-weight heparin (LMWH) and fondaparinux are commonly used for pharmacologic thromboprophylaxis [4]. Despite the introduction of newer anticoagulant agents, UFH is considered as the drug of choice for VTE prophylaxis in many populations. Subcutaneous (sc.) injection UFH is recommended for this purpose [6–11]. However, despite the use of the standard doses of anticoagulants, prevention of thrombosis is not desirable in some populations. Following changes in the pharmacokinetic and pharmacodynamic properties of sc. drugs in critically

ill patients, the adequacy of sc. administration of UFH or LMWH for thromboprophylaxis in these patients is questionable [6]. In some recent studies, significantly lower anti-Xa levels have been detected following single daily dose of sc. injection of enoxaparin in critically ill patients with normal renal function [11–16]. Robinson *et al.* reported that critically ill patients required higher doses of sc. enoxaparin to reach adequate anti-Xa levels [12,13].

Heparin exerts many of nonanticoagulant especially anti-inflammatory actions beyond its major anticoagulant effects [17–19]. Association between thrombosis pathogenesis and inflammatory conditions has been reported. Also inflammatory biomarkers have been proposed for prediction of thrombosis events. These biomarkers are D-dimer, P-selectin, inflammatory cytokines including IL-10 and fibrin monomers [20–22]. In this study, effects of different methods of UFH administration; sc. versus continuous intravenous

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infusion (CI) as thromboprophylaxis on the serum levels of thrombosis biomarkers have been compared in critically ill patients.

Methods

This open-labeled randomized clinical trial was conducted in the general intensive care unit (ICU) of Imam Khomeini hospital complex, a tertiary teaching hospital affiliated to Tehran University of Medical Sciences (Tehran, Iran) from March 2014 to the end of May 2015. The study protocol was approved by the ethics committee of Tehran University of Medical Sciences and was registered in the Iranian Registry of Clinical Trials (IRCT 201402113449N14). All patients or responsible family member signed informed consent form of the study.

At the beginning of the trial, 76 patients were screened for inclusion and exclusion criteria of the study. Patients older than 18 years, admitted to the ICU and who had indication of pharmacological thromboprophylaxis, were assessed for eligibility at the time of admission to the ICU.

Patients with confirmed thrombosis, thrombocytopenia (platelet count $<50,000/\text{ml}$), abnormal coagulation tests (international normalized ratio [INR] ≥ 1.5 or partial thromboplastin time [PTT] ≥ 40 s), active bleeding, active gastrointestinal ulcers, bleeding history in the previous 3 months were excluded. Moreover, conditions which affect immune system responses including autoimmune diseases, immunosuppressive or anti-inflammatory drug therapy also considered as exclusion criteria. Due to release of P-selectin from the platelets during hemodialysis and following acute myocardial infarction, these patients were excluded from the study [25–27].

Patients' physiological status was assessed based on the acute physiology and chronic health evaluation (APACHE II), simplified acute physiology score and sequential organ failure assessment scores at the time of admission to the ICU. Well's score is a clinical model for predicting probability of deep vein thrombosis (DVT) in a patient with symptoms of DVT [23]. In our study, this score was used for assessment of the likelihood of presence of DVT at the time of ICU admission. Patients with scores of <2 and ≥ 2 were considered unlikely and likely to have DVT at the time of admission to the ICU, respectively. Risk of bleeding also was calculated at this time [24]. Also all patients were followed for clinical signs and symptoms of thrombosis or bleeding during the study phase. Baseline and daily hemodynamic parameters and laboratory data including cell blood count and coagulation tests (prothrombin time [PT], PTT and INR) were considered for detection of thrombocytopenia and probable heparin-induced thrombocytopenia.

Patients with inclusion criteria of the study were randomly (based on the simple randomization method) assigned to intravenous CI or multiple sc. injections of UFH. Patients in the sc. and CI groups received UFH with dose of 5000 units subcutaneously three-times per day and 625 IU/h, respectively.

Demographic, clinical and laboratory data of the patients were extracted from their medical records. Included patients were followed for 7 days. Serum levels of hsCRP, IL-10 and P-selectin were considered as the thrombosis biomarkers.

For measurement of serum levels of the biomarkers, 5 ml venous blood sample was collected from each patient at the time of ICU admission and days 3 and 7 of hospitalization. Blood samples were kept for 30 min at the room temperature and then were centrifuged at 2500 RPM for approximately 20 min. The tubes containing serum were stored at -70°C until time of analysis.

ELISA was used for quantitative detection of serum levels of P-selectin (Bioassay Technology laboratory, Shanghai, China), IL-10 (Bioassay Technology laboratory) and hsCRP (Diagnostics Biochem Canada Inc. Ontario, Canada).

Assay procedures for measuring serum levels of P-selectin and IL-10 were similar. After serial dilution of the standard solutions and samples, they were added to the wells that were coated with P-selectin/IL-10 monoclonal antibody. Then streptavidin horseradish peroxidase was added to each well and incubated on a plate shaker for 60 min at 37°C . After washing the well five times, chromogen A and B solutions were added. Following an apparent color change, the stop solution terminated the ongoing reaction. As final step, the blank wells (which contained nothing but chromogen A and B solutions) were used to calibrate the spectrophotometer at 450 nm wavelength. According to the standard concentrations and the corresponding optical density values, the standard curve linear regression was calculated. The biomarkers levels were calculated by applying the optical density values of the samples on the regression equation.

For measuring serum hsCRP levels, the pretreated samples (diluted 1:20 with calibrator A), calibrators and control solutions along with assay buffer were added to the wells and incubated on a plate shaker for 30 min at room temperature. Each well was washed three times and then conjugated solution was added. The well was incubated for 15 min again and then was washed in the same manner of previous step. In next step tetramethylbenzidine (TMB) solution was added to the each well and once again was incubated for 15 min. Stopping solution was added and the plate

was read at 450 nm wavelength. Levels of hsCRP in the samples were calculated based on the standard curve linear regression equation.

Duration of ICU stay and mortality rate was considered as secondary outcomes of the study.

Statistical analysis

Statistical analyses were performed using the SPSS software (version 16.0; SPSS Inc., IL, USA). The Kolmogorov–Smirnov test was used to assess the normal distribution of all analyzed variables. Continuous variables were presented as mean with standard deviation (SD), and median with interquartile range boundaries when the data were not normally distributed. Continuous variables were compared between intervention groups using student's *t* or Mann–Whitney *U* test. Categorical variables were expressed through frequency in percentage and were compared between two groups using a chi-square test. Paired sample *t*-test and independent sample *t*-test were used for comparison of numeric variables between the groups. Changes in the levels of biomarkers during the study phase (days 0, 3 and 7) were assessed using repeated measurement ANOVA. *p*-value less than 0.05 was considered as statistically significant.

Results

From 63 patients who were recruited, 50 patients completed the trial. In the CI and sc. groups, six and seven patients respectively were discharged from the ICU within 7 days (Figure 1).

Demographic and clinical characteristics of the patients have been shown in Table 1. From all participants, 60 and 56% in the CI and sc. groups were male, respectively. Most of the included subjects were admitted to the ICU following surgery (62%), trauma (20%) and medical complications (18%). No statistically significant difference was detected between the groups regarding the demographic and clinical characteristics (Table 1).

The probability of thrombosis at the time of ICU admission, according to the Wells score, was comparable between the groups. The mean $\pm$ SD Wells score of the patients were 1.92 ± 0.49 and 1.80 ± 0.50 ; $p = 0.39$ in CI and sc. groups, respectively. Also mean $\pm$ SD bleeding score of the patients were 5.50 ± 1.47 and 5.02 ± 2.19 ; $p = 0.36$ in CI and sc. groups, respectively.

Cardiovascular agents (including α/β -blocker, ACEIs, ARBs, TNG, antiarrhythmias), antibiotics, antipsychotics, antidepressants, benzodiazepines, anticonvulsants, narcotics and acid suppressants were common concomitant drugs (Table 2).

During the study period, no thrombosis, thrombocytopenia or heparin-induced thrombocytopenia episode was detected. Also no bleeding or symptoms of

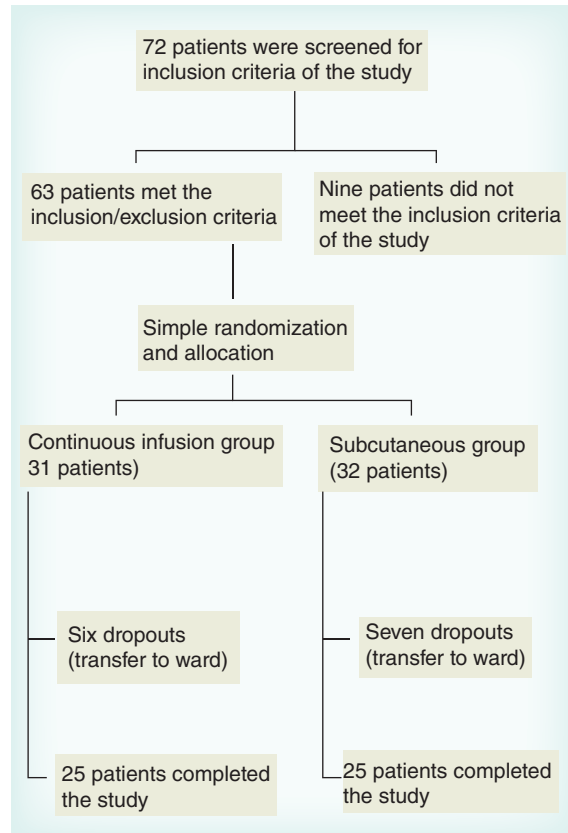


Figure 1. Consort flowchart of the study.

VTE was observed.

Change in the serum level of hsCRP during the study period has been evaluated by one-way repeated measures ANOVA. Mauchly's test confirmed the assumption of sphericity, $\chi^2(2) = 2.58$; $p > 0.274$. The change was not statistically significant within the groups ($F[2, 92] = 0.003$; $p = 0.997$). However, a significant difference in the serum levels of hsCRP was detected between the groups, $p < 0.001$. The serum levels of hsCRP were significantly lower in sc. than CI group at the all times of assessment ($F[1, 46] = 39.03$; $p < 0.001$) (Table 3).

For evaluating change in the serum level of P-selectin during the study one-way repeated measures ANOVA was considered. Mauchly's test of sphericity indicated that the assumption of sphericity had been violated, $\chi^2(2) = 11.94$; $p = 0.003$. A repeated measures ANOVA with a Greenhouse–Geisser correction showed that mean serum P-selectin levels did not differ significantly between the time points ($F[1.633, 78.41] = 0.067$; $p = 0.903$). Also the route of UFH administration had no significant effect on the level of serum P-selectin ($F[1, 48] = 2.36$; $p = 0.131$) (Table 4).

The same analysis was conducted to evaluate changes in the IL-10 serum levels during the study. The assumption of sphericity had been vio-

Table 1. Demographic and clinical characteristics of patients.

Characteristic	Continuous infusion (n = 25)	Subcutaneous injection (n = 25)	p-value
Age (years)	60.00 ± 19.21	56.61 ± 16.24	0.24 [†]
Sex (male)	15 (60)	14 (56)	0.50 [‡]
Weight (kg)	69.00 ± 19.84	65.02 ± 13.32	0.41 [†]
APACHE II score	15.96 ± 6.17	13.99 ± 5.11	0.17 [†]
SOFA score	6.28 ± 2.73	4.96 ± 2.71	0.10 [†]
SAPS II score	34.80 ± 11.35	31.67 ± 10.92	0.12 [†]
PTT (s); at time of ICU admission	28.34 ± 4.97	28.88 ± 8.18	0.78
PTT (s); mean during the study phase	30.11 ± 8.34	31.11 ± 9.07	0.83
Well's score	1.92 ± 0.49	1.80 ± 0.50	0.397 [†]
Diagnosis:			0.14 [‡]
– Medical	7 (28)	4 (16)	
– Surgery	13 (52)	16 (64)	
– Trauma	5 (20)	5 (20)	
Bleeding score	5.50 ± 1.47	5.02 ± 2.19	0.37 [†]
Atorvastatin administration:			0.51 [†]
– 20 mg	1 (4)	2 (8)	
– 40 mg	1 (4)	0 (0)	
Length of ICU stay (days)	17.52 ± 13.863	16.80 ± 15.877	0.86 [†]
Length of hospital stay (days)	25.56 ± 15.04	23.32 ± 15.21	0.59 [†]
Death	3 (12)	5 (20)	0.35 [‡]

Data have been presented as mean ± standard deviation or number (%) as indicated.
[†]Independent samples t-test.
[‡]Chi-square.
 APACHE II: Acute physiology and chronic health evaluation II; ICU: Intensive care unit; PTT: Partial thromboplastin time; SAPS II: Simplified acute physiology score II; SOFA: Sequential organ failure assessment.

lated by Mauchly's Test of Sphericity, $\chi^2 (2) = 7.88$, $p = 0.019$. Following repeated measures ANOVA with a Greenhouse–Geisser correction, it was shown that the serum level of IL-10 did not change significantly in the time points of the study ($F [1.71, 75.37] = 0.830$; $p = 0.424$). Also changes were not significant between CI and sc. groups ($F [1, 44] = 1.55$; $p = 0.219$) (Table 5).

Discussion

Over recent years, assessment of nonanticoagulant effects of heparin and related molecules has been considered in several experimental and clinical studies. Heparin exerts many of its nonanticoagulant actions through binding to the proteins such as chemokines and growth factors. However, exact mechanism of anti-inflammatory effect of heparin is not well known [28].

Endogenous heparin has a physiological role in regulating the inflammatory responses, through binding and

neutralization of the inflammatory mediators. Adherence of inflammatory cells to vascular endothelium and subsequent diapedesis into tissues are important parts of an inflammatory response. Heparin is effective in modulation of interaction between leucocytes and the vascular endothelium. This effect inhibits the recruitment of inflammatory cells to the tissues [29,30,18]. Changes in the pharmacokinetic behaviors of UFH or LMWHs in critically ill patients due to impaired sc. blood flow following vasopressor therapy [15], edema [16], alteration of heparin protein binding and change in heparin metabolism due to systemic inflammation and organ dysfunction [31–34] can affect efficacy of heparin as thromboprophylaxis in critically ill patients.

Intravenous UFH has been used in patients following bariatric surgery as an alternative method for thromboprophylaxis. This approach was associated with a lower rate of VTE without increasing adverse major bleeding events [7–10].

Table 2. Concomitant medications during the trial.

Drug category	Continuous infusion (n = 25), n (%)	Subcutaneous injection (n = 25), n (%)	p-value
Cardiovascular	12 (48)	10 (40)	0.38 [†]
Antibiotics	21 (84)	21 (84)	0.64 [†]
Narcotics	17 (68)	16 (64)	0.50
Psychiatrics and neurologic	8 (32)	6 (24)	0.37
Stress ulcer prophylaxis	25 (100)	25 (100)	–

[†]Chi-square.
 –: No statistics are computed because partial thromboplastin time is a constant.

Safety of CI of UFH has been evaluated in critically ill patients. In a single-blinded study, critically ill patients were randomized to receive either 5000 units of sc. UFH three-times a day or CI of UFH titrated to an activated partial thromboplastin time of 40–45 s. In this study, no major adverse event was detected in the both groups of patients [11].

Several biomarkers have been introduced as predictive factors of DVT. Role of P-selectin in the pathogenesis of DVT has been evaluated. Elevated serum soluble P-selectin level increased during acute phase of venous thrombosis and defined as a risk factor for VTE [35].

Some studies reported direct correlation between increased serum level of P-selectin and DVT in medical, surgical and cancer patients [36–39]. A raised serum soluble P-selectin level was defined as an indicator of generalized hypercoagulation state [37].

Anyhow, evaluating role of serum P-selectin level beside other predisposing factors of thrombosis to determine sensitivity and specificity of this biomarker in prediction of DVT is essential issue. Wang *et al.* prospectively evaluated role of serum P-selectin and D-dimer levels for the diagnosis of postoperative splenic or portal vein thrombosis. In this study, serum D-dimer and P-selectin levels had a significant positive predictive value in patients with thrombosis [40]. In Romacciotti *et al.* study serum soluble P-selectin level in combination with the wells score defined as an appropriate predictor of DVT. In this study, a high wells score (≥ 2) and serum P-selectin level above 90 ng/ml proposed for diagnosis of DVT with a positive predictive value of 100%. Interest-

ingly, considering serum P-selectin level ≤ 60 ng/ml (as cut-off point) and wells score < 2 , excluded the diagnosis of DVT with a negative predictive value of 96% [41].

However in Shi *et al.* study, correlation between serum level of P-selectin and DVT was not found. In this study, serum P-selectin level was measured in 91 patients following primary total hip arthroplasty. Change in the serum P-selectin level was not different in patients with and without DVT [42].

IL-10, a multiple functional cytokine with immunoregulatory effect, is another known biomarker evolved in the thrombosis pathogenesis [43]. Relationship between serum IL-10 level and thrombosis has been evaluated in few studies. Experimental data indicated that during acute phase of thrombosis, serum level of IL-10 was decreased and supplementation with IL-10 in a dose- and time-dependent manner declined the inflammatory and thrombotic cascades [44]. Association between IL-10 -1082A/G polymorphism and risk of DVT also has been shown [45]. However, there are few clinical evidences regarding correlation between serum level of IL-10 and thrombosis. In Poredos *et al.* study, serum IL-10 level proposed as an important clinical predictor of thrombosis in patients with idiopathic venous thrombosis. Included patients in this study had decreased levels of IL-10 and increased levels of proinflammatory cytokines [46].

The serum level of IL-10 in surgical patients suffered from DVT was significantly lower than those without postoperative DVT. It was proposed that increased serum level of IL-10, reduced inflammatory mediators

Table 3. Comparison of serum hsCRP levels between the groups at different times of the study.

Day	Continuous infusion (n = 25)	Subcutaneous injection (n = 25)	p-value [†]
0	11.123 $\pm$ 1.126	8.109 $\pm$ 1.589	< 0.001
3	10.797 $\pm$ 2.401	8.435 $\pm$ 1.594	< 0.001
7	11.072 $\pm$ 2.120	8.192 $\pm$ 1.617	< 0.001

For between groups comparison based on repeated measure analysis of variance, $p < 0.001$. For within groups comparison (repeated measure analysis of variance), $p = 0.997$. Values are presented as mean $\pm$ standard deviation.
[†]For between the groups comparison at different days (*post hoc* analysis).

Table 4. Comparison of serum P-selectin levels between the groups at different times of the study.			
Day	Continuous infusion (n = 25)	Subcutaneous injection (n = 25)	p-value [†]
0	7.88 ± 3.83	6.99 ± 4.31	0.442
3	8.04 ± 2.78	6.47 ± 3.55	0.089
7	7.95 ± 2.76	6.97 ± 4.13	0.331
For between groups comparison based on repeated measure analysis of variance, p = 0.131. For within groups comparison (repeated measure analysis of variance), p = 903. Values are presented as mean ± standard deviation.			
[†] For between the groups comparison at different days (<i>post hoc</i> analysis).			

and to some extent prevent development of thrombotic events. IL-10 may be a potential target for prevention and treatment of postoperative DVT especially in abdominal malignancies [47].

The role of CRP in the pathogenesis of venous thrombosis is controversial. In two large prospective studies, serum CRP level was not predictive factor for development of thrombosis [48,49]. This result also was confirmed in other studies [50–52,20]. Vormittag *et al.* detected a higher basal serum hsCRP levels in patients with spontaneous VTE compared with healthy controls, but it was not an independent risk factor for VTE [53]. In Hald *et al.* prospective study, serum level of hsCRP did not show any correlation with future development of VTE [54].

Conclusion

Our study was first randomized clinical trial that compared effects of CI versus sc. administration of UFH on the thrombosis biomarkers in critically ill patients. We did not detect any thrombosis episode or major adverse event of UFH in included patients. Changes in the thrombosis biomarkers were comparable in both sc. and CI groups during the study period. Serum level of hsCRP as an inflammatory biomarker was significantly different between the groups at the time of ICU admission. The results of the study may be influenced by this different inflammatory patients’ condition. Small sample size and short duration of patients’ follow-up were other major limitations of our study. Future randomized controlled trials with larger sample size and enough duration of patients’ follow-up should be considered. Also effects of adjusted heparin doses based on the targeted activated-PPT or anti-Xa factor

on the thrombosis biomarkers may be considered in future studies.

Future perspective

Future randomized controlled trials with larger sample size and enough duration of patients’ follow-up should be considered. Also effects of adjusted heparin doses based on the targeted activated-PPT or anti-Xa factor on the thrombosis biomarkers may be considered in future studies.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Table 5. Comparison of serum IL-10 levels between the groups at different times of the study.			
Day	Continuous infusion (n = 25)	Subcutaneous injection (n = 25)	p-value [†]
0	198.72 ± 111.39	208.66 ± 80.78	0.803
3	196.01 ± 83.33	237.32 ± 76.98	0.135
7	184.22 ± 97.20	214.49 ± 91.56	0.286
For between groups comparison based on repeated measure analysis of variance, p = 0.219. For within groups comparison (repeated measure analysis of variance), p = 0.424. Values are presented as mean ± standard deviation.			
[†] For between the groups comparison at different days (<i>post hoc</i> analysis).			

Executive summary

In this study, effects of different methods of unfractionated heparin (UFH) administration (subcutaneous [sc.] vs intravenous continuous infusion [CI]) as thromboprophylaxis on the serum levels of thrombosis biomarkers have been compared in critically ill patients.

Method

- Patients with inclusion criteria of the study were randomly (based on the simple randomization method) assigned to CI or multiple sc. injections of UFH.
- Included patients were followed for 7 days.
- Serum levels of hsCRP, IL-10 and P-selectin were considered as the thrombosis biomarkers.

Results

- Change in the serum level of hsCRP during the study period was not statistically significant within the groups (F [2, 92] = 0.003; p = 0.997). However, a significant difference in the serum levels of hsCRP was detected between the groups, p < 0.001.
- Route of UFH administration had no significant effect on the level of serum P-selectin (F [1, 48] = 2.36; p = 0.131).
- Serum level of IL-10 did not change significantly in the time points of the study (F [1.71, 75.37] = 0.830; p = 0.424). Also changes were not significant between CI and sc. groups (F [1, 44] = 1.55; p = 0.219).

Conclusion

- No any thrombosis episode or major adverse event of UFH has been detected.
- Changes in the thrombosis biomarkers were comparable in both sc. and CI groups during the study period.

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• of interest; •• of considerable interest

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