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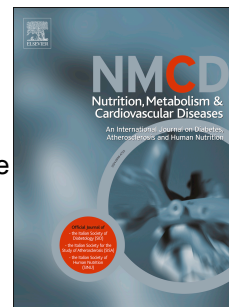
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Association between antithrombin and mortality in patients with COVID-19. A possible link with obesity.

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Abstract

Background and aims. Despite anticoagulation, usually with heparin, mortality for thromboembolic events in COVID-19 remains high. Clinical efficacy of heparin is due to its interaction with antithrombin (AT) that may be decreased in COVID-19. Therefore, we correlated AT levels with outcomes of COVID-19.

Methods and Results. We recruited 49 consecutive patients hospitalized for COVID-19. AT levels were significantly lower in 16 non-survivors than in 33 survivors (72.2 ± 23.4 versus $94.6\pm 19.5\%$; $p=0.0010$). A multivariate Cox regression analysis showed that low AT (levels below 80%) was a predictor of mortality (HR:3.97; 95%CI:1.38 to 11.43; $p=0.0103$). BMI was the only variable that showed a significant difference between patients with low and those with normal AT levels (32.9 ± 7.9 versus $27.5\pm 5.9\%$; $p=0.0104$). AT levels were significantly lower in obese patients than in subjects with normal weight or overweight (77.9 ± 26.9 versus 91.4 ± 26.9 versus $91.4\pm 17.1\%$; $p=0.025$). An inverse correlation between AT levels and BMI was documented ($r:-0.33$; $p=0.0179$).

Conclusions. Our data first suggest that AT is strongly associated with mortality in COVID-19. In addition, AT may be the link between obesity and a poorer prognosis in patients with COVID-19. Other studies should confirm whether AT may become a prognostic marker and a therapeutic target in COVID-19

Highlights

Reduced Antithrombin is associated to mortality in COVID-19

Reduced Antithrombin is associated to need for mechanical ventilation in COVID-19

Reduced Antithrombin is associated to obesity in COVID-19

Reduced antithrombin is associated to heparin resistance

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Introduction

Coronavirus Disease 2019 (COVID-19) is characterized by an elevated risk for thromboembolic events that can lead to death [1]. This risk is attributed to a procoagulant state due to hyperinflammation [1,2]. So prophylactic doses of heparin are recommended in all the hospitalized patients and a therapeutic anticoagulation, mainly with heparin, is used when thromboembolic events occur [1,2]. However, despite anticoagulation, mortality in COVID-19 remains high [1,2,3,4]. The clinical efficacy of heparin is mainly due to its interaction with antithrombin (AT), a powerful natural anticoagulant [5]. Hyperinflammation can markedly decrease AT levels and can reduce its physiological actions [5]. This suggests that anticoagulation may be ineffective in patients with low AT levels (heparin resistance) [5]. Nevertheless, whether COVID-19 affects AT levels and whether there is an association between AT and outcomes of COVID-19 remains unclear, considering that specific studies were never carried out. Therefore, we evaluated AT levels in patients hospitalized for COVID-19 and correlated AT levels with the outcomes of COVID-19.

Methods

We retrospectively enrolled 49 consecutive patients admitted to our hospital for Severe Acute Respiratory Syndrome–Coronavirus 2 (SARS-CoV2) pneumonia. Pneumonia was documented by Chest Computed Tomography and infection was established by Real Time-Polymerase Chain Reaction. On admission, AT was measured with a chromogenic method (HemosIL, Werfen, Barcelona, Spain) together with all the other laboratory tests routinely made in patients hospitalized for COVID-19. All the patients received standard treatment for SARS-CoV2 pneumonia according to the protocol of our hospital: hydroxychloroquine, lopinavir/ritonavir, antibiotics (doxycyclin and ceftriaxone) and a prophylactic dose of enoxaparin. Other tailored treatments, including tocilizumab, therapeutic anticoagulation, steroids and/or AT concentrate, were made on the basis of clinical courses. The study was carried out according to the ethical standards of the institutional research committee and with the 1964 Helsinki declaration.

In this retrospective study of prospectively collected data the primary outcome was death. A combined outcome of death or need for mechanical ventilation in survivors was taken into account.

Statistical analysis. Univariate analysis was carried out by using Student t-test to find significant differences and the exact Fisher's test for frequency comparisons. To find differences among three groups ANOVA was used. The Pearson's correlation was used to analyze statistical associations. Nonnormally distributed variables were log-transformed before the analysis. Survival curves were estimated by the Kaplan-Meier test and compared by the Mantel log-rank test. The effect of the variables with a $p < 0.1$ at the univariate analysis on the primary outcome and on the combined outcome was tested in a multivariate Cox regression analysis. Variables were dichotomized before the analysis. A $p < 0.05$ was considered statistically significant.

Results

Table 1 shows that non-survivors had troponin levels significantly higher and AT levels and oxygen saturation (SaO₂) significantly lower than survivors. The Kaplan Meier method showed that AT was associated with a higher mortality (log-rank test: 5.5398; $p = 0.0186$) (Figure 1 Panel A). A multivariate analysis with the primary outcome as the dependent variable was exploited. The following variables were tested and dichotomized before the analysis: history of diabetes (yes or not), BMI (< 25 or ≥ 25), high-sensitivity troponin: < 20 or ≥ 20 pg/ml), Oxygen Saturation (SaO₂) (< 93 or $\geq 93\%$), AT (< 80 or $\geq 80\%$). A multivariate Cox regression analysis showed that low AT (levels below 80%) was a predictor of mortality (HR: 3.97; 95%CI: 1.38 to 11.43; $p = 0.0103$) together with a history of diabetes (HR: 4.40; 95%CI: 1.30 to 1.82; $p = 0.0167$) and a normal BMI (HR: 0.22; 95%CI: 0.05 to 0.90; $p = 0.0351$). When the combined outcome of death or need for mechanical ventilation in survivors was considered, the difference in AT levels between the patients with the combined outcome ($n = 24$) and survivors who did not require mechanical ventilation ($n = 25$) remained significant (73.7 ± 20.6 versus $100.4 \pm 17.5\%$; $p < 0.0001$). The Kaplan Meier method showed that AT was associated with the combined outcome (log-rank test: 5.8272; $p = 0.0158$) (Figure 1 Panel B). The variables with $p < 0.1$ at the univariate analysis were history of diabetes, BMI, troponin, ScO₂ and AT and therefore were tested in a multivariate analysis. A multivariate Cox regression analysis showed that low AT was the only predictor of the combined outcome (HR: 2.56; 95%CI: 1.13 to 5.81; $p = 0.0238$). Table 2 reports the patients stratified by normal/low AT levels; as shown, the two groups did not significantly differ in any variable except for BMI that was significantly higher in patients with reduced AT levels. When the patients

were divided into three subgroups according to the presence of normal weight (BMI<25; n=11), overweight (BMI 25-29.9; n=23) and obesity (BMI \geq 30; n=15), AT levels were significantly lower in obese patients (77.9 \pm 26.9%) than in subjects with normal weight (91.4 \pm 26.9%) and overweight (91.4 \pm 17.1%): ANOVA $p=0.025$. An inverse correlation between AT levels and BMI was documented in the whole population ($r=-0.33$; $p=0.0179$). Mortality and the percentage of patients with the combined outcome were significantly higher in patients with low than in those with normal AT levels.

Discussion

We found AT levels below the lower limit of the normal range in 30.6% of our patients hospitalized for COVID-19; among them, 66.7% died. Among survivors with low AT levels, 80% of them required mechanical ventilation. Our preliminary data first show that low AT levels seem to be associated with a high mortality and need for mechanical ventilations in patients with COVID-19. This independent association may be at least partially due to the fact that anticoagulation, mainly with heparin, may be ineffective [5]. Several studies showed that thrombosis, both in the venous and arterial circulations, seems to be the main cause of death in patients with COVID-19 [1,2]. On the other hand, microthrombi were regularly found within small lung arteries of all the patients who died because of COVID-19 [6]. The high thromboembolic risk is due to a procoagulant state that is caused by an excessive inflammatory response through several mechanisms [1,2]. Abnormal coagulation parameters [3], presence of lupus anticoagulant [7] and antiphospholipid antibodies [8] have been described in patients with severe COVID-19. To prevent and treat thromboembolic events in patients with COVID-19 prophylactic or therapeutic anticoagulation is recommended [1,2]. However, despite the anticoagulation, mortality in COVID-19 is very high [1,2,3,4]. Heparin is usually used for anticoagulation in these patients [1,2,3,4]. The clinical efficacy of heparin is mainly due to its interaction with AT [5], but severe inflammatory states markedly decrease AT levels because of a reduced synthesis [5]. Then, inflammation promotes degradation of elastase from activate neutrophils and causes a consumption due to thrombin generation [9]. In addition, the function of AT depends on glycosaminoglycans of the endothelial surface, but their synthesis is reduced by inflammatory

mediators [10]. Therefore, reduced levels and impaired function of AT may explain why heparin is ineffective in some patients with COVID-19.

Our study shows that in hospitalized patients with severe COVID-19 the proportion of subjects with low AT levels is quite high, but this finding is in agreement with previous studies [3,11,12]. Tang found that AT levels were lower in non-survivors than in survivors even if difference did not attain statistical significance [3]. Panigada found low mean AT levels in 11 patients admitted to the Intensive Care Unit [11]. Ranucci observed that among 16 patients admitted to Intensive Care Unit 25% of them had AT levels below the lower limit of the normal range [12]. Taken together, these data suggest that a high proportion of patients with COVID-19 can have low AT levels. A recent study suggests that acutely low AT levels are even common in severe forms of COVID-19 [13]. However, it is unclear why the acute decrease in AT regards only a proportion of patients. Inflammation can acutely and markedly reduce AT levels [5], but our data did not show any difference in inflammatory factors or other variables between patients with low and those with normal AT levels. BMI is the only variable that shows a significant difference between the two groups. In particular, BMI was significantly higher in patients with reduced AT levels. In addition, we have also found an inverse correlation between AT levels and BMI in the whole population. To better evaluate the relationship between BMI and AT in patients with COVID-19, they were stratified according to the presence of normal weight, overweight and obesity; AT levels were significantly lower in obese patients than in the other two subgroups. It is important to remember that obesity is a recognized powerful risk factor for a worse prognosis in COVID-19 [14]. Our data seems to suggest that AT may be the link between obesity and poorer prognosis, including death [14]. It is of interest to note that obesity is characterized by chronic inflammation and a pro-thrombotic state [15], but decreased AT levels were not clearly documented [16]. However, obese patients seem to be more prone to a marked reduction in AT levels when hyperinflammation of COVID-19 occurs. The reasons remain unclear.

In our study troponin levels were significantly higher in non-survivors, even if they did not maintain the statistical significance in multivariate analysis. This may be due to the relatively small study population. However, elevated troponin levels are expression of cardiac impairment and therefore they may be reliable

predictors of worse outcomes of COVID-19, as recently documented [17]. This may imply that troponin should be considered as a predictor of survival in severe COVID-19 together with other biological parameters [17], including AT.

If confirmed, our findings can have important clinical implications. First, AT may become a strong predictor of worse outcomes of COVID-19 and therefore it may be used in the stratification of the risk for thrombosis and death. AT levels should be measured on admission and monitored over time, as they can be reduced by heparin itself [5]. In addition, patients with low AT levels should be treated with AT concentrate to face heparin resistance [5,18]. On the other hand, AT has also anti-inflammatory properties and therefore it may reduce hyperinflammation due to COVID-19 [11]. We used AT concentrate together with heparin in patients with low AT and worsening disease progression, but mortality was high. Specific studies should evaluate whether an earlier use of AT concentrate may improve outcomes. Alternatives to AT concentrate may be fresh frozen plasma [18] or anticoagulants other than heparin [13]. However, we cannot exclude that also patients with normal AT levels may have heparin resistance due to an impaired function of AT, as inflammation acts not only on the synthesis but also on the function [5].

The main limitations of our study are the relatively small population and the retrospective design. However, even if it is a retrospective analysis all data are available and no patient was lost to the follow-up.

In conclusion, our data first suggest that AT is strongly associated with mortality in COVID-19. In addition, AT may be the link between obesity and a poorer prognosis in patients with COVID-19. Other studies should confirm whether AT may become a prognostic marker and a therapeutic target in severe COVID-19

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Table 1. features of the whole study population and of survivors and non-survivors

variable	Total patients (n=49)	Survivors (n=33)	Non-survivors (n=16)	p-value
Age (years)	69.4±14.8	67.1±14.5	74.1±14.9	0.1245
Males (%)	49.0	45.4	56.2	0.5512
History of Diabetes (%)	20.4	12.1	37.5	0.0596
History of Hypertension (%)	34.7	33.3	37.5	0.9989
History of CVD (%)	16.3	12.1	25.0	0.4105
History of Lung Disease (%)	16.3	12.1	25.0	0.4105
BMI	29.1±7.0	28.0±6.4	31.5±7.8	0.0975
eGFR (ml/min)	70.6±27.0	70.4±25.3	70.8±31.1	0.9667
CRP (mg/L)	122.5±85.5	116.1±79.8	135.8±97.6	0.9245
D-dimer (ng/ml)	1848.7±5949.4	1436.8±3902.6	2698.2±8923.6	0.6387
High-sensitivity Troponin (pg/ml)	20.1±25.2	14.1±17.7	32.4±33.5	0.0246
AT (%)	87.3±23.2	94.6±19.5	72.2±23.4	0.0010
Prothrombin time (sec)	13.1±3.1	12.8±2.3	13.8±4.3	0.3035
activated partial-thromboplastin time (sec)	32.6±5.4	33.3±5.5	31.2±5.2	0.2207
SaO ₂ (%)	90.5±5.8	92.6±4.3	87.6±7.2	0.0043
Lactate dehydrogenase (U/L)	344.7±161.4	326.6±149.3	382.0±183.3	0.3669

AT: antithrombin; SaO₂: oxygen saturation; BMI: body mass index. CVD: cardiovascular Disease; CRP: C-reactive Protein

Table 2. features and outcomes of the patients stratified by normal/low antithrombin levels.

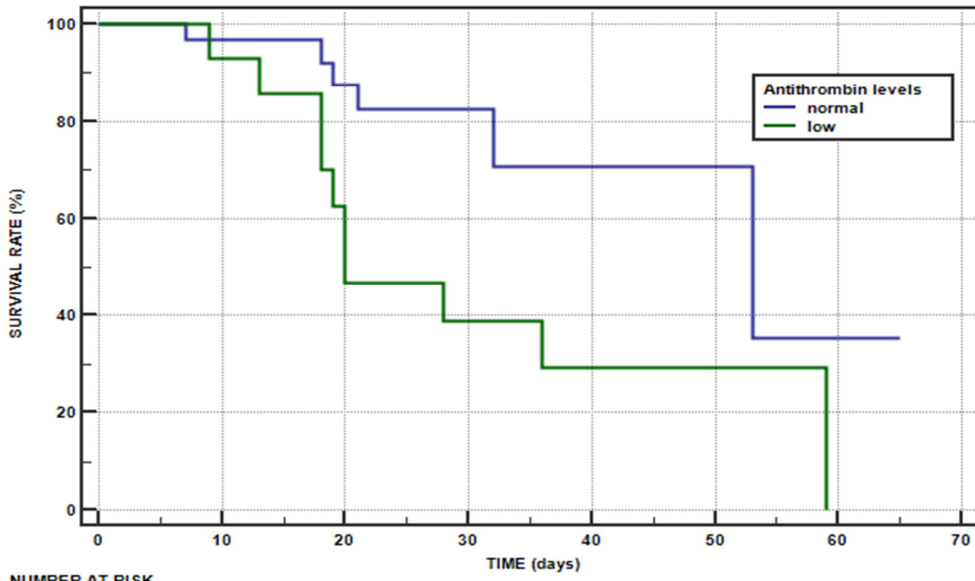
variable	Total patients (n=49)	Patients with normal AT (n=34)	Patients with low AT (n=15)	p-value
Age (years)	69.4±14.8	71.0±13.8	65.7±16.9	0.2507
Males (%)	49.0	47.1	53.3	0.7624
History of Diabetes (%)	20.4	23.5	13.3	0.7021
History of Hypertension (%)	34.7	35.3	33.3	0.9999
History of CVD (%)	16.3	17.6	13.3	0.9898
History of Lung Disease (%)	16.3	17.6	13.3	0.9898
BMI	29.1±7.0	27.5±5.9	32.9±7.9	0.0104
eGFR (ml/min)	70.6±27.0	68.1±28.8	76.1±22.2	0.3448
CRP (mg/L)	122.5±85.5	121.7±83.8	124.4±92.3	0.8530
D-dimer (ng/ml)	1848.7±5949.4	2455.5±7084.2	433.3±375.2	0.7065
High-sensitivity Troponin (pg/ml)	20.1±25.2	18.4±25.1	23.8±25.9	0.1789
AT (%)	87.2±23.2	99.6±15.2	59.2±9.4	<0.0001
Prothrombin time (sec)	13.1±3.1	12.9±2.3	13.6±4.4	0.4948
activated partial- thromboplastin time (sec)	32.6±5.4	33.3±5.6	31.1±5.0	0.2050
SaO2 (%)	90.5±5.8	91.8±5.2	89.1±7.0	0.1342
Lactate dehydrogenase (U/L)	344.7±161.4	333.7±159.0	369.7±169.6	0.3794
Non-survivors (%)	32.6	17.6	66.7	0.0019
Survivors who required mechanical ventilation (%)	24.3	14.3	80	0.0076
Non survivors and survivors who required mechanical ventilation (%)	49.0	29.4	93.3	<0.0001

AT: antithrombin; SaO2: oxygen saturation; BMI: body mass index. CVD: cardiovascular Disease; CRP: C-reactive Protein

Figure 1. Kaplan–Meier survival curve according to normal/low antithrombin levels in patients hospitalized for COVID-19 (PANEL A) and Kaplan–Meier curve of the combined outcome of death or the need for mechanical ventilation in survivors according to normal/low antithrombin levels in patients hospitalized for COVID-19 (PANEL B)

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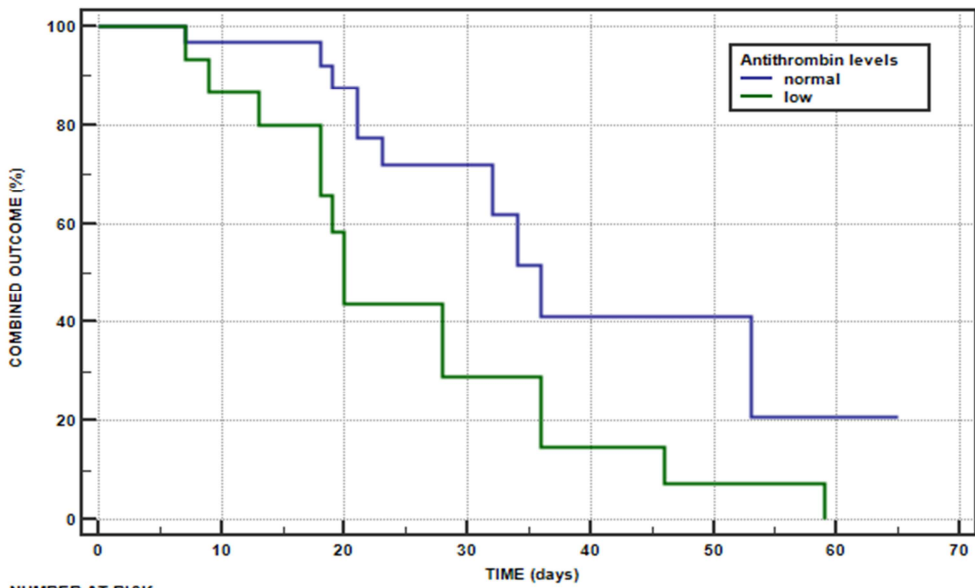
PANEL A



NUMBER AT RISK

Time (days)	0	10	20	30	40	50	60	70
normal	34	26	17	7	3	2	1	0
low	15	13	6	4	2	1	0	0

PANEL B



NUMBER AT RISK

Time (days)	0	10	20	30	40	50	60	70
normal	34	26	17	7	3	2	1	0
low	15	13	6	4	2	1	0	0

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