



## The effect of circulating cytokines on cardiovascular patients infected with the Coronavirus

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### ABSTRACT

The coronavirus illness (COVID-19) is caused by serious acute respiratory disorder coronavirus 2 (SARS-CoV-2), moreover known as the COVID-19 virus. After the first-ever reports of COVID-19 in December 2019, the malady spread quickly. In January 2020, the WHO announced the outbreak a Public Health Emergency of Worldwide Concern, and by March 2020, the WHO characterized the episode as a global widespread. The current study aimed to detect the effect of SARS-CoV-2 infection in heart patients and study their immune response by detecting the levels of some cytokines, which may end in a cytokine storm and may lead to death. In this study, one hundred-eight subjects were enrolled on two comparison case-control groups, the case group included 54 patients suffering from SARS-COV2, all were selected from those who were admitted to the Intensive Care Unit (ICU), and were diagnosed by a specialist physician with severe acute respiratory syndrome due to SARS-COV2 documented by Real-Time Polymerase Chain Reaction( RT-PCR ) besides other clinical and laboratory criteria in Marjan Medical City in Babylon province, AL-Amal Hospital for Communicable Diseases and AL-Hakeem Hospital, Najaf/Iraq, for a period from March 2022 to October 2022 to evaluate the role of some selected serological among patients with SARA-COV2. The control group in this study included 54 subjects, divided into three groups (Apparent Healthy, patients suffered from SARS-COV2, patients suffered from CVD). Blood samples were examined through immunological methods, and an enzyme-linked immunosorbent assay (ELISA) was adopted for the detection of the concentration of TNF- $\alpha$ , IL6, IL-10, IL-12 and CCL2. The immunological evaluation to clarify the theory of cytokines storm carried in the present study revealed that (TNF- $\alpha$ , IL6, IL-10, IL-12, and CCL2) for patients with COVID-19 and CVD was significantly higher than all the comparison group. The study reported that interleukin (6, 10, 12) and TNF-a are significantly increased in patients with covid19, CVD, and COVID-19 patients only, compared to healthy people. furthermore, IL-6 and IL-12 levels increased in patients with CVD only when compared to healthy people. There is a significant increase in CCL2 in all study groups compared to healthy people who have lower levels and this study indicated that the infection with Covid disease was severe and critical in most patients with CVD. This increased the number of deaths among them.

**Keywords:** *patients, infected, coronavirus*

## INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-caused (COVID-19) pandemic has swept global since the first case discovered in the Chinese city of Wuhan in December 2019. Over 25 million people in more than 200 countries have been infected, and over 840,000 people have died as a result of it, according to the COVID-19 Dashboard published on August 31st, 2020 by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University( Hopkins ., 2020).

Severe acute respiratory syndrome - coronavirus-2 is an enveloped, non-segmented, single-stranded, positive sense RNA virus (+ssRNA). The genome size ranges from (27 to 32 kb) a cap structure at the 5' ends followed by a reader sequence of about 70 bases, several open reading frames (ORFs) coding various proteins, and a non-translated region including a poly-A sequence at the 3' ends (Khailany et al., 2020).

The clinical presentation of SARS-CoV-2 begins after 14 days of exposure, however symptoms often appear after surrounding 5 days and in 97.5% of people, symptom onset is within 11 days (Lauer et al., 2020). Respiratory failure is the leading cause of death in patients with COVID-19 infection, but symptoms of cardiovascular disease could contribute in total mortality (Huang et al., 2020). Prolonged high levels of cytokines, characterized as the cytokine storm, may exacerbate systemic immune disorder (Chen et al.,2020). Patients with SARS-COV2 may develop severe complications due to cytokine storm(Xu et al.,2020).

Interleukin-6 is a polypeptide with a molecular weight of 22-27 kD that is secreted by activated monocytes, macrophages, fibroblasts, adipocytes, and endothelial cells in response to numerous stimuli such as TNF-, IL-1b, bacterial endotoxins, physical activity, and oxidative stress (Jones et al.,2001). Furthermore, in patients with severe COVID-19, IL-6 overexpression can cause fever, vascular leakage, anaemia, cardiomyopathy, acute kidney injury (AKI), and myocardial dysfunction ( Zhou et al.,2020).

Interleukin-10 is a key immunoregulatory cytokine that inhibits inflammatory cytokine generation and interferes with antigen

presentation by lowering MHC class II expression on antigen-presenting cells (Moore et al.,2001). Besides, increased IL-10 expression is associated with a poor prognosis in COVID-19 (Han et al., 2020).

Interleukin-12 (IL-12) is a newly discovered cytokine with a distinctive heterodimeric structure made up of the polypeptides p40 and p35; the majority of IL-12 is produced by macrophages and monocytes following the stimulation; it is a multifunctional cytokine that regulates T lymphocytes and NK cells in a number of different ways (Trinchieri,1995). Higher levels of IL-12 (p70) have been associated with severe COVID-19 (Liu et al., 2021).

Endothelial cells, fibroblasts, epithelial cells, smooth muscle cells, mesangial cells, astrocytes, monocytes, and microglia all produce CCL2, also known as monocyte chemoattractant protein-1 (MCP-1) (Barna et al.,1994).Increased CCL2 in COVID-19 is related with both respiratory failure and extrapulmonary symptoms. Increased CCL2, together with IL-1, IL-6, TNF-a, MMP-8, and ICAM-1, might increase the permeability of the blood-cerebrospinal fluid barrier, enhancing inflammatory infiltration (Gram et al.,2014).

TNF- is a highly strong proinflammatory cytokine with a wide range of effects. Many inflammatory conditions, including cytokine release syndrome, have been linked to significant elevations. Serum TNF- levels were shown to be higher in SARS-COV2 patients, with the increase being more pronounced in the more severe cases (Huang, et al.,2020).

## MATERIALS AND METHODS

This study was designed according to case-control groups, The sum of the samples was one hundred-eight subjects, the group included fifty-four patients who suffered from SARS-COV2 with CVD and were diagnosed by a specialist physician with severe acute respiratory syndrome due to SARS-COV2 documented by RT-PCR beside other clinical criteria and laboratory tests in Marjan Medical City in Babylon province and AL-Amal Hospital for Communicable Diseases and AL-Hakeem Hospital, Najaf/Iraq. for a

period from march 2022 to October 2022. The control (comparison group) in this study included 54 subjects, divided into three groups ( Apparent Healthy, patients who suffered from SARS-COV2, and patients who suffered from CVD ) .

Coronavirus patients' blood samples were collected. TNF-, IL6, IL-10, IL-12, and CCL2 concentrations in blood samples were determined using immunological techniques and enzyme-linked immunosorbent assay (ELISA). 5ml of blood samples in plain tubes were left at 37 °C for 30 minutes before being centrifuged at 3000 rpm for 15 minutes. The clot was then removed, and the residuals were centrifuged at 3000 rpm for 10 minutes. Following that, the sera were aspirated with an automatic micropipette and transferred to two clean test tubes for serological testing. Labels were attached to each test tube, which was subsequently stored and frozen at -20°C for later serological testing to assess the concentrations of TNF-, IL6, IL-10, IL-12 and CCL2 using the ELISA kit (MELSIN, China) . The test was carried out by the manufacturer's instructions on the kit.

## RESULT AND DISCUSSION

### *Interleukin 6 (IL-6)*

Displays in Table (1) that the mean of IL-6 for patients with COVID-19 and CVD was  $8.08 \pm 0.978$  ng/mL is significantly higher than for patients with CVD and control subjects ( $7.23 \pm 0.420$  ng/mL and  $6.82 \pm 0.580$  ng/mL, respectively). Patients with Covid-19 are significantly higher than patients with CVD, and control subjects, the mean of IL-6 in patients with Covid-19 was  $7.91 \pm 0.774$  ng/mL compared to  $7.23 \pm 0.420$  ng/ml, and  $6.82 \pm 0.580$  ng/mL for patients with CVD, and control subjects respectively. While there are no significant differences between patients with COVID-19 and CVD, and patients with Covid-19 (P .value >0.05).

The results of this study agreed with Lubrano and Balzan, (2020) who revealed the high levels of IL-6 in patients with cardiovascular diseases and Covid-19. The present results found that the IL-6 level in patients with COVID-19 and CVD (G1) are significantly higher than in control subjects. These results correspond with research by

Nguyen et al., (2022), who reported that Covid-19 patients with increased levels of IL-6 had a greater risk of experiencing serious adverse cardiac events than individuals with normal levels of IL-6 (P <0.0001, odds ratio [OR] = 5.91, 95% confidence interval [CI] = 2.65-14.11). The scientific explanation for the relationship between interleukin-6 and patients with cardiovascular disease and Covid-19 are due to COVID-19 is caused by the SARS-CoV-2 virus, which can trigger an inflammatory response in the body, leading to the production of cytokines such as IL-6. This can result in a condition known as a cytokine storm, which can damage organs and tissues, including the heart (Montazersaheb et al., 2022).

In this study, the IL-6 level in patients with CVD (G3) is significantly higher than in control subjects. This result is in agreement with the study findings conducted by (Patterson et al., 2010), which found that IL-6 retained a significant association with coronary heart diseases. Also, previous studies indicated that high levels of IL-6 concentrations in patients with unstable angina( Lubrano et al., 2005).

The scientific explanation for the relationship between interleukin-6 and patients with cardiovascular disease (G3) is based on previous studies where it was reported that the association between cytokines, monocyte movement in the intima, and atheroma formation is now well understood. The atheroma attracts T-cells, mast cells, and other inflammatory cells to the intima (Hansson, 2005) and produces several factors (Riley et al., 2008) that break down collagen, causing the fibrous cap to shrink and become unstable (Libby, 2001). Atherosclerotic plaque instability has been observed to be correlated with IL 6 and IL 8 cytokine levels(IuI et al., 2012).

The present results found that the IL-6 level in patients with COVID-19(G2) is significantly higher than in control subjects. These results with Lu et al., (2021) revealed patients with COVID-19 had a high level of IL-6 compared to control subjects. Possible explanations for the rise in interleukin-6 may be due to either the SARS-CoV-2 virus, which causes COVID-19, can directly infect and damage cells in the body,

including immune cells. This can trigger the release of IL-6 and other pro-inflammatory cytokines as part of the immune response (Tang et al., 2020), or in severe cases of COVID-19, the immune system can become dysregulated,

leading to a condition known as a cytokine storm. This is characterized by an excessive release of cytokines, including IL-6, which can cause widespread inflammation and tissue damage (Tang et al., 2020).

**TABLE 1:** Levels of IL-6 in the study group and control groups.

IL-6		Mean± SD	Range (Min-Max)
Patients With COVID-19(With CVD) (G1)		8.08±0.978 a	6.54-9.98
Patients With COVID-19 (G2)		7.91±0.774 a	6.53-9.34
Patients With CVD (G3)		7.23±0.420 b	6.54-8.15
Control Subjects (G4)		6.82±0.580 c	5.62-7.70
Least Significant Difference (LSD)	Absolute Mean Difference (AMD)		
0.442	(G1 Vs. G2) = 0.17	AMD < LSD	P. value >0.05 NS
	(G1 Vs. G3) = 0.85	AMD > LSD	P. value <0.05 HS
	(G1 Vs. G4) = 1.26	AMD > LSD	P. value <0.05 HS
0.407	(G2 Vs. G3) = 0.68	AMD > LSD	P. value <0.05 HS
	(G2 Vs. G4) = 1.09	AMD > LSD	P. value <0.05 HS
	(G3 Vs. G4) = 0.41	AMD > LSD	P. value <0.05 HS

Different Letters denote the significant Difference at P . value <0.05

**Interleukin 10 (IL-10)**

Shows table (2) that the mean of IL-10 for patients with COVID-19 and CVD(G1) was 206.01±63.96ng/mL is significantly higher than for patients with CVD(G3) and control subjects(G4) (178.20±20.22ng/mL and 165.04±19.4 ng/mL, respectively). Patients with Covid-19 (G2) are significantly higher than control subjects(G4), the mean of IL-10 in patients with Covid-19 was 184.32±23.28 ng/mL compared to 165.04±19.4 ng/mL control subjects. While there are no significant differences between patients with COVID-19 and CVD, and patients with Covid-19 (P .value >0.05). Furthermore, no found significant differences were between patients with CVD and control subjects.

The present results found that the IL-10 level in patients with COVID-19 and CVD is significantly higher than in control subjects. These results with research by Ma et al., (2021)revealed high levels of IL-10 in patients with cardiovascular diseases and Covid-19 compared with the control subjects.

The results indicate that the IL-10 level in patients with COVID-19 is significantly higher than in control subjects. This result is in agreement with the study findings done byLu et al., (2021), which found that patients with COVID-19 had a high level of IL-10 compared to control subjects. Possible explanations for the rise in interleukin-10 may be due to, either IL-10 may be produced in response to the excessive inflammatory response seen in severe cases of COVID-19, as a way to dampen down the immune response and prevent tissue damage, or COVID-19 may induce the production of IL-10 as a way to evade the immune system and promote viral persistence (Islam et al., 2021).

In this study, there are no significant differences between patients with CVD and control subjects. These results are inconsistent with the study findings done by Tabrez et al., (2017), which found a high level of IL-10 in patients with CVD compared to patients without CVD. Overall, non-significant differences in IL-10 levels between patients with CVD and a control group could be due to a range of factors, including patients with

CVD are a heterogeneous group, and IL-10 levels may vary depending on the specific type and severity of CVD. If the patient group includes a mix of different types and stages of CVD, this can obscure any true differences in IL-10 levels.

**TABLE 2:** Levels of IL-10 in the study group and control groups.

IL-10	Mean± SD	Range (Min-Max)	
Patients With COVID-19(With CVD) (G1)	206.01±63.96 a	143.48-365.00	
Patients With COVID-19 (G2)	184.32±23.28 a b	160.87-250.00	
Patients With CVD (G3)	178.20±20.22 b c	143.47-220.00	
Control Subjects (G4)	165.04±19.41 c	134.78-210.00	
Least Significant Difference (LSD)	Absolute Mean Difference (AMD)		
25.936	(G1 Vs. G2) = 21.69	AMD < LSD	P. value >0.05 NS
	(G1 Vs. G3) = 27.81	AMD > LSD	P. value <0.05 HS
	(G1 Vs. G4) = 40.97	AMD > LSD	P. value <0.05 HS
14.083	(G2 Vs. G3) = 6.12	AMD < LSD	P. value >0.05 NS
	(G2 Vs. G4) = 18.91	AMD > LSD	P. value <0.05 HS
	(G3 Vs. G4) = 13.16	AMD < LSD	P. value >0.05 NS

**Interleukin 12(IL-12)**

Reveals in Table (3) that the mean of IL-12 for patients with COVID-19 and CVD(G1)was 7.92±0.86ng/mL is significantly higher than for patients with CVD (G3)and control subjects(G4) (7.51±0.63ng/mL and 6.84±0.51ng/mL, respectively). Patients with Covid-19(G2) are significantly higher than control subjects(G4), the mean of IL-12 in patients with Covid-19 was 7.74±0.62 ng/mL compared to 6.84±0.51 ng/mL control subjects. While there are no significant differences between patients with COVID-19 and CVD(G1) and patients with Covid-19(G2) (P .value >0.05). Furthermore, no found significant differences were between patients with Covid-19 and patients with CVD.

The present results found that the IL-12 level in patients with COVID-19 and CVD is significantly higher than in control subjects. These results with the studies by Mokhtari et al., (2020) revealed the high levels of IL-12 in the patients with cardiovascular diseases and Covid-19 compared with the control subjects .

The results indicate that the IL-12 level in patients with COVID-19 is significantly higher

than in control subjects. This result is in agreement with the study findings done by Gajovic et al., (2023),which found that patients with COVID-19 had a high level of IL-12 compared to control subjects.

In COVID-19, IL-12 is thought to be elevated as a result of the body's immune response to the SARS-CoV-2 virus. IL-12 is produced by dendritic cells and macrophages in response to viral infection, and it helps to activate natural killer cells and T cells, which are important components of the immune response. However, excessive production of IL-12 can also contribute to the development of cytokine storm, a severe immune response that can lead to lung damage and other complications in COVID-19 patients (Costela-Ruiz et al., 2020).

In CVD, IL-12 is believed to be elevated as a result of chronic inflammation. Inflammatory cells such as macrophages and T cells produce IL-12, which contributes to the development of atherosclerosis (hardening of the arteries) and other cardiovascular complications. Elevated levels of IL-12 have been found in the blood vessels and plaques of patients with CVD, and it

is thought to play a role in the progression of the disease (Van der Heijden et al., 2019).

**TABLE 3:** Levels of IL-12 in the study group and control groups.

IL-12	Mean± SD	Range (Min-Max)	
Patients With COVID-19(With CVD) (G1)	7.92±0.86 a	6.00-9.96	
Patients With COVID-19 (G2)	7.74±0.62 a b	6.48-8.84	
Patients With CVD (G3)	7.51±0.63 b	6.80-9.32	
Control Subjects (G4)	6.84±0.51 c	6.01-7.90	
Least Significant Difference (LSD)	Absolute Mean Difference (AMD)		
0.401	(G1 Vs. G2) = 0.18	AMD < LSD	P. value >0.05 NS
	(G1 Vs. G3) = 0.41	AMD > LSD	P. value <0.05 HS
	(G1 Vs. G4) = 1.08	AMD > LSD	P. value <0.05 HS
0.397	(G2 Vs. G3) = 0.23	AMD < LSD	P. value >0.05 NS
	(G2 Vs. G4) = 0.90	AMD > LSD	P. value <0.05 HS
	(G3 Vs. G4) = 0.67	AMD > LSD	P. value <0.05 HS

**Tumour Necrosis Factors-a (TNF-a)**

Shows table(4) that the mean of TNF-a for patients with COVID-19 and CVD (G1) was 11.22±2.21 ng/mL is significantly higher than for patients with CVD(G3) and control subjects (G4) (9.42±2.47 ng/mL and 9.04±1.22 ng/mL, respectively). Patients with Covid-19 (G2) are significantly higher than patients with CVD and control subjects, the mean of TNF-a in patients with Covid-19 was 11.05±1.70 ng/mL. While there are no significant differences between patients with COVID-19 and CVD, and patients with Covid-19 (P .value >0.05). Furthermore, no found significant differences were between patients with CVD and control subjects.

In this study, patients with COVID-19 and CVD have a high level of TNF-a significantly higher than control subjects. These findings were consistent with (Song et al., 2020), which discovered that tumor necrosis factor- (TNF-) levels surpassed normal limits, and that TNF- levels were statistically higher in the myocardial injury group (with Covid-19) than in the non-myocardial injury group (without Covid-19).

The results indicate that the TNF-a level in patients with COVID-19 is significantly higher than in control subjects. This result is in

agreement with the study findings done by Gajovic et al., (2023), which found that patients with COVID-19 had a high level of TNF-a compared to control subjects. TNF-alpha (TNF-a) is a pro-inflammatory cytokine that plays a crucial role in the immune response to infections and other inflammatory conditions (Bradley, 2008).

In COVID-19, TNF-a is released by immune cells in response to the viral infection. The excessive production of TNF-a in COVID-19 has been linked to a severe inflammatory response known as a cytokine storm, which can cause damage to multiple organs and lead to a poor prognosis. TNF-a has also been implicated in the pathogenesis of acute respiratory distress syndrome (ARDS), a severe complication of COVID-19 (Giamarellos-Bourboulis et al., 2020).

In CVD, TNF-a is produced by various cell types, including endothelial cells, smooth muscle cells, and macrophages. Elevated levels of TNF-a have been observed in patients with atherosclerosis, heart failure, and other cardiovascular conditions. TNF-a can contribute to the development and progression of CVD by promoting inflammation, oxidative stress, endothelial dysfunction, and

thrombosis. TNF-a can also cause cardiomyocyte heart muscle damage and dysfunction ( Shechter apoptosis (programmed cell death), leading to et al., 2014) .

**TABLE 4:** Levels of TNF-a in the study group and control groups.

TNF-a	Mean± SD	Range (Min-Max)	
Patients With COVID-19(With CVD) (G1)	11.22±2.21 a	2.58-13.63	
Patients With COVID-19 (G2)	11.05±1.70 a	8.52-15.76	
Patients With CVD (G3)	9.42±2.47 b	2.57-12.78	
Control Subjects (G4)	9.04±1.22 b	7.67-11.50	
Least Significant Difference (LSD)	Absolute Mean Difference (AMD)		
1.111	(G1 Vs. G2) = 0.17	AMD < LSD	P. value >0.05 NS
	(G1 Vs. G3) = 1.8	AMD > LSD	P. value <0.05 HS
	(G1 Vs. G4) = 2.18	AMD > LSD	P. value <0.05 HS
1.253	(G2 Vs. G3) = 1.63	AMD > LSD	P. value <0.05 HS
	(G2 Vs. G4) = 2.01	AMD > LSD	P. value <0.05 HS
	(G3 Vs. G4) = 0.38	AMD < LSD	P. value >0.05 NS

**CCL2**

Shows table(5) that the mean of CCL2 for patients with COVID-19 and CVD(G1) was 53.41±15.509 ng/mL is significantly higher than control subjects(G4) (38.48±6.055 ng/mL). Patients with Covid-19(G2) are significantly higher than control subjects(G4), the mean of CCL2 in patients with Covid-19(G2) was 53.00±10.188 ng/mL. Patients with CVD (G3) are significantly higher than control subjects(G4), the mean of CCL2 in patients with CVD(G3) was 50.28±13.889 ng/mL compared to control subjects(G4) (38.48±6.055 ng/mL). While there are no significant differences between patients with COVID-19 and CVD(G1) and patients with Covid-19(G2) (P .value >0.05). Furthermore, no found significant differences were between patients with CVD (with Covid-19) (G1) and patients with CVD(G3).

CCL2, also known as monocyte chemoattractant protein-1 (MCP-1), is a chemokine that plays a key role in immune cell recruitment and inflammation (Zlotnik & Yoshie, 2012). The results indicate that the CCL2 level in patients with Covid-19 is significantly higher than in control subjects. These findings are consistent with the previous study results conducted by

Hussein, (2021),who reported that elevated serum levels of CCL2 have an important role in the severity of COVID-19 infection.

The results indicate that the CCL2 level in patients with CVD is significantly higher than in control subjects. This result is in agreement with the study findings done by McDermott et al., (2005), who reported that there was a high level of CCL2 in patients with Myocardial Infarction. In patients with CVD, elevated levels of CCL2 may be due to the activation of inflammatory pathways that are common in atherosclerosis and other cardiovascular conditions. CCL2 may contribute to the recruitment of monocytes to atherosclerotic lesions, which can exacerbate inflammation and lead to plaque instability and rupture (Kim et al., 2020).

In patients with COVID-19, elevated levels of CCL2 may be due to the activation of the immune system in response to the viral infection. CCL2 may contribute to the recruitment of monocytes and other immune cells to the lungs, which can lead to inflammation and tissue damage. Additionally, CCL2 may play a role in the cytokine storm that is associated with severe COVID-19, in which an overactive immune

response can cause widespread inflammation and organ damage (Ranjbar et al., 2022).

**TABLE 5:** Levels of CCL2 in the study group and control groups.

CCL2	Mean± SD	Range (Min-Max)	
Patients With COVID-19(With CVD) (G1)	53.41±15.509 a	20.00-81.26	
Patients With COVID-19 (G2)	53.00±10.188 a	40.87-69.57	
Patients With CVD (G3)	50.28±13.889 a	30.44-81.01	
Control Subjects (G4)	38.48±6.055 b	27.43-46.09	
Least Significant Difference (LSD)	Absolute Mean Difference (AMD)	CCL2	
7.182	(G1 Vs. G2) = 0.41	AMD < LSD	P. value >0.05 NS
	(G1 Vs. G3) = 3.13	AMD < LSD	P. value >0.05 NS
	(G1 Vs. G4) = 14.93	AMD > LSD	P. value <0.05 HS
7.054	(G2 Vs. G3) = 2.72	AMD < LSD	P. value >0.05 NS
	(G2 Vs. G4) = 14.52	AMD > LSD	P. value <0.05 HS
	(G3 Vs. G4) = 11.80	AMD > LSD	P. value <0.05 HS

### CONCLUSION

The study reported that interleukin (6, 10, 12) and TNF-a are significantly increased in patients with covid19, CVD, and COVID-19 patients only, compared to healthy people. furthermore, IL-6 and IL-12 levels increased in patients with CVD only when compared to healthy people. There is a significant increase in CCL2 in all study groups compared to healthy people who have lower levels and this study indicated that the infection with Covid disease was severe and critical in most patients with CVD. This increased the number of deaths among them.

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