Original Article

Association of Interleukin-2 (IL-2) Levels With Acid-Fast Bacillus (AFB) Sputum Conversion In Drug-Resistant Pulmonary Tuberculosis Patient

Gede Sasmika Suwandi[1], Yani Jane Sugiri[1], Tri Wahju Astuti[1], Harun al Rasyid[2]

[1]Department of Pulmonology and Respiratory Medicine Faculty of Medicine, Universitas Brawijaya, dr. Saiful Anwar General Hospital, Malang
[2]Department of Public Health Sciences and Preventive Medicine Faculty of Medicine, Universitas Brawijaya, Malang

Corresponding Author:
Gede Sasmika Suwandi | Department of Pulmonology and Respiratory Medicine Faculty of Medicine, Universitas Brawijaya, dr. Saiful Anwar General Hospital, Malang| sasmikasuwandi@gmail.com

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Abstract

Background: Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) which can be found on multiple organs, but mostly infect the lung. AFB smear is used to diagnose and evaluate therapy response, to show bacterial burden and the patient's infectious status. Combination of antituberculosis drugs for a long period of time could cause poor compliance and increased risk of resistance. Mtb infection induce immune response and release cytokines, one of which is interleukin-2 (IL-2) that regulate T lymphocyte cell. Higher IL-2 levels is found on patient with high bacterial burdern. This study aims to see the assosciation between IL-2 levels before and 30 days after DR-TB treatment, to see pre and post-treatment IL-2 levels with conversion of AFB smear, and correlation between pre-treatment IL-2 levels and AFB smear.

Method: This was a cohort prospective study at RSUD dr Saiful Anwar Malang. Thirty nine DR-TB patients underwent AFB smear before and 30 days after treatment, while IL-2 levels was also measured by Enzym-Linked Immunosorbent Assay (ELISA) technique.

Results: The levels of IL-2 before treatment was significantly higher compared to 30 days after treatment (P<0.001). There was no significant IL-2 levels difference between conversion and non-conversion group (P=0.23), and a weak, not-significant correlation between IL-2 levels and AFB smear (r=0.28; P=0.06).

Conclusion: The levels of IL-2 was significantly higher before than after treatment and it cannot be used to determine the positivity of acid-resistant bacilli smears in the sputum of patients with DR-TB. The limited timeframe and biomarker in this study raise the possibility to observe IL-2 as well as other biomarkers after intensive phase of TB treatment for future studies.

Keywords: drug resistance tuberculosis (DR-TB) , interleukin-2 (IL-2), AFB smear

INTRODUCTION

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. Despite several trials to implement a tuberculosis elimination strategy, Indonesia has high incidence of the case. World Health Organization (WHO) report on 2019 estimated tuberculosis death rate in Indonesia is 35 per 100,000 population, meaning that around 93,000 people died from tuberculosis (TB) in 2018. The number of tuberculosis cases has increased sharply since 2017 after efforts were done to track tuberculosis cases in hospitals. The number of tuberculosis case reports in 2018 was 565,869 cases, meanwhile, the number of TB case findings in 2019 Global TB Report was 570,289 cases.[1]

One of the burdens of tuberculosis is drug resistance. Rifampicin is an anti-tuberculosis drug that works by binding the RNA-beta polymerase subunit, thereby inhibiting the bacterial mRNA elongation. 96% of rifampicin resistance cases are caused by mutations in the "hot-spot region" by 81-bp spanning codons 507-533 in the rpoB gene.[2]

Drug-resistant tuberculosis (DR-TB) is still a problem frequently found in the world and also in Indonesia. Indonesia is included in high-burden countries for multidrug resistant tuberculosis (MDR-TB) cases. According to WHO, MDR-TB cases are about 3.3% of new cases. The proportion of MDR-TB mostly came from patients who had previously received anti-tuberculosis therapy, which counts to around 20%.[3]

In 2015, it was reported that there were 9.6 million new TB cases around the world, with 480,000 cases being MDR-TB with mortality rate of 190,000. Globally, in 2016, around 4.1% of new cases and 19% of previously treated cases were diagnosed with MDR-TB or rifampicin-resistant tuberculosis (RR-TB).[3]

The incidence of MDR-TB in 2015 was estimated to be around 600,000, with 82% (490,000) of cases being MDR-TB and the rest were rifampicin-resistant TB. The countries with leading MDR-TB cases were China, India, and Russia. Mortality caused by MDR-TB were around 240,000 cases. Meanwhile, the proportion for extensively drug resistant tuberculosis (XDR-TB) was around 6.2%. In Indonesia alone, there were 2.8% of new MDR-TB cases, with 16% of cases being patients who have been treated for TB. There were around 12 patients per 100,000 population who suffered from MDR-TB with the percentage of MDR-TB being 68%.[3,4] Based on the data from Prasetya, the proportion of new DR-TB patients at Saiful Anwar Hospital Malang reached 21 new patients each month.[5]

There are several cytokines which had a role in tuberculosis immunopathogenesis, i.e., interleukin-2 (IL-2), IL-4, and IL-10. Interleukin 2 is a cytokine that has been studied to be a recombinant for adjunctive immunological therapy against tuberculosis. Using this study, we had baseline data on IL-2 levels in new DR-TB cases and after 1 month of treatment. This is because the activation of IL-2 by T cells will activate immune response. Before performing as a

treatment, it is necessary to detect IL-2 levels in TB patients, especially in patients with drug resistance.[6]

There are previous studies assessing IL-2 levels in active tuberculosis patients. When it was compared between early diagnosis and after 2 months of antituberculosis drug therapy, IL-2 levels were found to be significantly higher in early detection. In addition, IL-2 levels on patients with TB were found to be higher than healthy patients.[6]

Conversion of sputum smear also determines whether the disease is infectious, the potential of transmission from TB patients to the community and resistance to antituberculosis drugs leads to poorer outcomes.[7,8] A study by Kim et al, showed that at 4 weeks of treatment, the sputum conversion rate was 52.7% in drug-sensitive-TB group and 45.7% in MDR and XDR-TB groups. Based on a study by Prasetya in 2019 at Saiful Anwar Hospital Malang, BTA sputum conversion time was obtained the most within the first month by 35.3%.[5]

This study will assess IL-2 levels in newly diagnosed TB patients with drug resistance, prior to and 1 month after DR-TB treatment.

METHOD

This is a cohort prospective study, conducted in March-July 2020. This study was conducted on pulmonary TB patients who were treated at MDR-TB Polyclinic at dr. Saiful Anwar Malang. The inclusion criteria in this study were patients diagnosed with rifampicin-resistant pulmonaryTB, aged 18-65 years, who were willing to participate in the study and signed an informed consent. Patients who had received anti-MDR-TB drugs for ≥1 month, patients with Human Immunodeficiency Virus (HIV) and pregnant women were not included in this study. The minimum sample size was 38. Samples were obtained by means of consecutive sampling that fulfilled the criterias. In this study, 39 subjects who met the inclusion and exclusion criteria were subjected to Acid Fast Bacillus (AFB) sputum smear and IL-2 levels measurement on day 0 and day 30 after receiving MDR-TB treatment (Shorter Regimen/Longer Regimen).

Data processing and analysis were carried out using IBM SPSS version 22.0 software. The data is tested for normality first to determine whether the data distribution is normal or not. The relationship between IL-2 levels before treatment and sputum smear result will be analyzed using Person correlation test if data with normal distribution or Spearman correlation test if data with abnormal distribution. The differences in IL-2 levels on day 0 and day 30 based on the conversion status of AFB sputum smear were analyzed using independent T-test if data with normal distribution or Mann-Whitney if data with abnormal distribution. Differences in IL-2 levels before and after treatment were analyzed using paired T-test if data with normal distribution or Wilcoxon if data with abnormal distribution.

The degree of confidence used is 95% or α=0.05

RESULTS

This research was conducted from March to July 2020, at the MDR-TB Polyclinic at dr. Saiful Anwar Malang. There were 39 subjects who met the inclusion and exclusion criteria and were willing to take part in the study by signing an informed consent.

The subjects of this study were rifampicin-resistant pulmonary TB patients aged 18-65 years with 44.85±12.85 years. Most of the research subjects were male which counted to 25 subjects and female which counted to 19 subjects. The research subjects had a mean body mass index (BMI) of 18.67±3.08. Most of the subjects (26 people) had previous history of pulmonary TB. Based on data on comorbid diseases, there were 13 subjects with DM and 3 with cardiovascular diseases as shown in Table 1.

Based on the clinical data recapitulation of DR-TB patients on treatment at dr. Saiful Anwar Malang, obtained results as shown in Table 2. Based on clinical symptoms, the majority of complaints were coughing for more than 2 weeks, as found in 26 subjects (66.7%). The main complaints of study subjects, ranged from the most to the least frequency, were prolonged cough, hemoptysis, shortness of breath, weakness and fever.

Table 1. Characteristic of the Study Subjects

Characteristics	N (%)	Mean±SD	CI 95%
Age		44,85±12,85	40,68-49,01
<20 years old	1 (2,6)		
20-29 years old	6 (15,4)		
30-39 years old	5 (12,8)		
40-49 years old	10 (25,6)		
50-59 years old	11 (28,2)		
60-69 years old	6 (15,4)		
Sex			
Male	20 (51,3)		
Female	19 (48,7)		
BMI		18,67±3,08	17,68-19,67
Underweight (<18,5)	18 (46,1)		
Normal (18,5-24,9)	20(51,30)		
Overweight (25-26,9)	1 (2,6)		
Comorbid			
DM	13 (33,3)		
Cardiovascular Disease	3 (7,7)		
No comorbid	26 (66,7)		
History of TB			
Yes	26 (66,7)		
No	13 (33,3)		

Based on laboratory findings for DR-TB patients on treatment at dr. Saiful Anwar Malang, chest X-rays were classified into minimal lesion, moderate lesion and far advance lesion.

Table 2. Clinical Characteristics of the Subjects

Clinical Characteristics	N	%
Chief Complaints		
Chronic cough	26	66,7
Bloody cough (Hemoptysis)	7	17,9
Shortness of breath	3	7,7
Weakness	2	5,1
Fever	1	2,6
TCM Result		
Detected very low	4	10,3
Detected low	4	10,3
Detected medium	22	56,4
Detected high	9	23
Pre-Treatment AFB smear		
Negative	11	28,2
Scant	2	5,2
1+	11	28,2
2+	5	12,8
3+	10	25,6
CXR		
Minimal lession	1	2,6
Moderate lession	13	33,3
Far advance lession	25	64,1
AFB Conversion		
Positive	1	2,6
Negative	38	97,4

The most common findings in this study were far advance lession, as found in 25 people (64.1%). Based on rapid molecular assays, the subjects mostly belonged to MTB detected medium group, as found in 22 people (56.4%). Based on other tests such as AFB smear, the most common findings were consecutively negative, 1+, and 3+. This showed that the number of AFB identified from the patient's sputum vary.

IL-2 levels measured pre-treatment and 30 days after treatment were not normally distributed, thus, data transformation was performed with $\log_{10}$ function. IL-2 levels were examined using ELISA method and measured in units of pg/ml. Paired T-test was performed to see whether there was a significant difference between IL-2 levels before and 30 days after treatment. Subjects underwent DR-TB therapy regimen and regularly took the drug for 1 month. In this study, the range of IL-2 was found between 2.119 – 37.982 pg/mL. Analysis with paired T-test can be seen in Table 3. Paired T-test showed a significant decrease in IL-2 values before and 30 days after treatment.

Table 3. Paired T test to compare IL-2 levels before and after treatment

Characteristics	Mean±SD (pg/ml)	P
IL-2 before treatment	0,94 (0,25)	<0,001
IL-2 after 1 month of treatment	0,72 (0,24)	

Table 4. Correlation between Il-2 levels before and 30 days after treatment based on AFB conversion status

Characteristics	Mean±SD (pg/ml)	P	CI 95%
Before treatment			
Non-conversion (n=1)	0,64	0,23	-0,31 (-0,82-0,20)
Conversion (n=38)	0,95 (0,25)		
After treatment			
Non-conversion (n=1)	0,46	0,28	-0,27 (-0,77-0,23)
Conversion (n=38)	0,73 (0,24)		

To see the correlation between IL-2 levels before and 30 days after treatment with BTA conversion, the independent T-test was performed. Comparison of IL-2 levels was carried out in groups with positive and negative BTA conversion. The value of group with a positive BTA result was 1 person, while the value of groups with a negative BTA result was 38 people. The correlation between IL-2 levels before and 30 days after treatment with BTA conversion can be seen in Table 4.

The difference between pre-treatment IL-2 levels in the conversion and non-conversion groups was 0.27 pg/ml. Meanwhile, the difference between IL-2 levels 30 days after treatment in the conversion and non-conversion groups was 0.31 pg/ml. There was no significant difference in IL-2 levels between the conversion and non-conversion groups before and after treatment.

The correlation between pre-treatment IL-2 values and AFB smear was analyzed using the Spearman test. This test was conducted to see whether there was a linear correlation between IL-2 value and AFB smear. The analysis result obtained the value of r=0.28 and P=0.064.

There was a weak correlation between IL-2 levels and AFB smear ($0.2<r<0.4$). The value of r showed a positive correlation, meaning that the higher IL-2 value was, the more BTA could be found. The value of $P>0.05$ that indicated that the correlation between two variables is not statistically significant.

DISCUSSION

The average the subjects age in this study was within the productive range. Based on 2017 WHO report and 2016 Indonesian Minister of Health Regulation, the productive age group is has the highest TB incidence. This is due to high mobility and wide social contacts out of the house in this group. Apart from contacting the disease, this group also plays a role in TB transmission.[9]

In this age range, various diseases such as diabetes mellitus and HIV also begin to arose, thus, increases the risk of TB infection. A study conducted in Shenzhen, China, showed a significant increase in TB incidence annually from 2011 to 2016 within the >45 years age group.[9] Similar results were found in a study conducted in Guangzhou from 2007 to 2012. The incidence of TB significantly rises in the 45-64 years age group. The high TB incidence in this population can be caused by dense living environments, poor sanitation, low public health awareness, and limited access to health facilities.[10]

The number of male and female subjects in this study was nearly the same, viz, 20 male subjects and 19 females. Data from WHO 2017 showed that nearly 6 million men suffer from TB with a death rate of 840,000, while there are 3.2 million cases in women with a death rate of nearly 500,000. This is in accordance to a study by Paudel which stated that DR-TB patients were dominated by male population. The high rate of DR-TB infection in males was associated with a history of dropping out of

treatment, in which with males are more prone to than females.[11] The higher ration of male TB patients, especially in developing countries, is caused by various socioeconomic factors. Men's role is more likely to be the backbone of the family, thus, having a higher likelihood of being accepted into a work sector that is not well organized. Low health awareness and working hours which limits the time used to go to health facilities also played an important role in the high number of TB cases in men.[12] The ratio of male and female subjects in this study was 1:1. This is probably due to the small number of samples and the use of consecutive sampling method, therefore making this number not accurately describing the actual ratio in the community.

Based on the results of the study, it was found that 66.7% of DR-TB patients in this study did not have comorbidities, while 33.3% had DM and 7.7% had cardiovascular disorders. This is in accordance with the research conducted by Prasetya in 2019 in Malang, in which was found that DR-TB patients with comorbidities are 43.8%, 42.6% of which being DM and 1.2% of which being HIV. Another study found that the prevalence of MD-TB in DM patients was 36% and TB patients with DM had a risk of becoming DR-TB patients 8.6 times greater than those without DM. DM can increase the risk of failure or relapse in TB treatment, and in TB patients with DM there was a delay in sputum conversion.[13]

Normal BMI range in Asian population is 18.5-23 kg/m^2. The average BMI in this study was 18.67 kg/m^2. Even though this value is still within the normal range, it is still within the lower limit, thus, it can be assumed that the nutritional status of the patient tends to be below average or poor. There is a strong correlation between pulmonary TB infection and low BMI. A study conducted in Korea on 304,202 subjects found that the risk of TB infection decreased as BMI increased. This protective effect can be explained as release of proinflammatory and tumor necrosis factor (TNF) markers in patients who have more visceral fat which tends to be higher. The presence of proinflammatory markers acts as an immune mediator that protects against TB infection.[14] Low BMI are also associated with TB reinfection and higher mortality rates in TB-HIV patients.[15]

About 66.7% of subjects in this study had a previous history of pulmonary TB. A research by Rifat et al. showed that 54% of MDR-TB patients had previously been infected with TB. Most MDR-TB patients underwent treatment more than once.[16] Unregulated TB treatment can lead to increase of *Mycobacterium tuberculosis* resistance pattern. In addition, poor health care in inadequate health facilities and transmission in the community also increases the incidence of MDR-TB. Similar results were also obtained in a research conducted in 2019 by Prasetya in Malang, in which was stated that 47.1% of diagnosed DR-TB patients were relapse cases where the patients had previous history of pulmonary TB and had underwent treatment.[5]

Clinical symptoms of TB were divided into respiratory complaints, include chronic cough, hemoptysis, shortness of breath, and non-respiratory complaints, for instance fever and weakness. The most frequently complained symptom in this study was prolonged cough (66.7%) while the least one was fever (2.6%). These results are in concordance with a study conducted by Tolossa et al. in Ethiopia in 2013. Tolossa et al. found that 72.4% patients had complained chronic cough as the main symptom.[17]

In Prasetya's study, the patients mostly complained with productive cough (wet cough) of more than 2 weeks, which were found in all subjects (100%).[5] Generally, the symptoms of DR-TB were the same as drug-sensitive-TB, in which the most common symptom was chronic cough. Another study conducted by Sirait et al also showed that the most common complaint (88%) was chronic cough.[18] Other symptoms that might be present are shortness of breath, chest pain, bloody cough or hemoptysis, and systemic symptoms i.e., fever, chills, night sweats, fatigue or malaise, and weight loss.[19]

About 71.8% of subjects in this study had positive AFB smear result (scanty, 1+, 2+, 3+). Systematic review and meta-analysis conducted by Hermosilla S et al using 162,574 subjects from 14 countries showed a positive smear result in 52% of adult TB patients.[20] Patients with positive smear had a higher risk of transmission than patients with negative smear result. The high results in this study, more than expected, can be due to the high burden and rate of infection in Indonesia. In addition, 33.3% of patients in this study had diabetes mellitus. Patients with diabetes mellitus had a higher probability of having a positive smear result than patients without diabetes mellitus (OR=5.0; 95% CI=2.4-20.7; P<0.01).[21]

AFB conversion in this study was assessed after the subjects underwent treatment for 30 days. 97.4% of patients had negative AFB conversion while 1 (2.6%) had not. However, the patient still showed a conversion from +3 to +1. This result is different from a study conducted in Lianyungang, China in 92 patients with MDR-TB. The median of duration for AFB conversion to negative was 1 month, however, only 60% of patients had AFB conversion at the end of treatment in the second month. Patients who experienced conversion within 2 months of treatment had a better outcome than patients who did not experience conversion (OR=7.19, CI 95%=2.60-19.84). The sensitivity and specificity of the conversion results to predict the outcome were 67.6% (CI 95%=50.2-82.0) and 76.4% (CI 95%=32.0-65.6). Patients responsive to ofloxacin are known to show a better response to therapy.[22]

Another study conducted in Ethiopia in 235 MDR TB patients showed a higher conversion rate. The median duration required for conversion was 54 days. The number of patients who successfully experience conversion to negative at the end of the second month of treatment was 89.2%. Patients with TB-HIV or low BMI experienced a longer conversion time.[23]

The high rate of AFB conversion within 1 month in this study could be due to the high effectiveness of the drug regimen, the absence of HIV as a comorbid in the patients, and the BMI of patients who were still within normal range.

Radiological chest X-ray in this study showed that the most common lesions were far advanced lung lesions, followed by moderate and minimal lesions. Several studies of DR-TB patients showed similar results, in which the X-ray showed cavitas, fibrosis, consolidation, calcification, atelectasis, and bullae.[24] Another study by Prasetya in 2019 found that the majority of chest X-ray examination of DR-TB patients showed lung TB with extensive lesions (90.6%). The sensitivity and specificity of chest X-ray in diagnosing TB were 86% and 83%.[5] A study by Sihombing et al on DR-TB patients showed infiltrate on 81 subjects (92.25%) cavity on 15 subjects (17.65%) and pleural effusion on 11 subjects (12.94%). Chest imaging on TB patients can provide an overview of various kinds of lesions.[25]

The IL-2 values in this study were measured twice, viz. before and 30 days after treatment. Pre-treatment IL-2 had a higher mean value than after treatment IL-2. There was a significant difference between IL-2 before and 30 days after treatment ($P<0.001$).The IL-2 is a cytokine produced by Th0, Th1 and some Cytotoxic T Lymphocytes (CTL). These cytokines stimulate the growth of B, T and NK cells, which subsequently stimulate the immune system through proliferation, maintain T lymphocytes and differentiate naïve T lymphocytes into effectors and memory cells. They are essential for cellular immunity and granuloma formation in MTB infection.[26] A study conducted by Hur et al. showed a significant increase in IL-2 on active TB patients compared to healthy patients.[27] Similar results were found in the study conducted by Wang et al. on 215 subjects. The IL-2 values were significantly higher in the group with active pulmonary TB than in the inactive TB and control groups.[28]

There are several studies that further assess the role of IL-2 as a diagnostic tool. The IL-2 performed well in identifying TB and non-TB patients, however it still failed to differentiate active and latent TB.[29] The results in this study support the role of IL-2 as a diagnostic tool. The significant reduction in IL-2 after 30 days of treatment also suggests its potential as a tool for evaluation of therapy in the future.

As previously explained, IL-2 plays a role in stimulating the immune system and protecting against TB infection. Patients with TB infection had higher IL-2 values than the normal group. Higher IL-2 values were also found at the start of treatment and significantly reduced as treatment progressed. In this study, the independent T-test was performed to assess the difference in mean IL-2 values prior to and 30 days after treatment in the positive and negative AFB conversion groups. There is only 1 subject (2.6%) who did not experience AFB conversion to negative after 30 days of treatment. Because there was only 1 subject included in the non-conversion category, the results of

comparation test between the conversion and non-conversion groups were less relevant. The log_{10} value of IL-2 prior to treatment was higher by 0.31 pg/ml (CI 95%=-0.82-0.20; P=0.23) in the conversion group than in the non-conversion group. The log_{10} IL-2 value 30 days after treatment was also higher by 0.27 pg/ml (95% CI=-0.77-0.23, P=0.28) in the conversion group than in the non-conversion group.

The results of this study differ from the results of research conducted by Luo et al. The aforestated research found that the group of patients who did not undergo conversion had significantly higher sIL-2R values than the conversion group. The sIL-2R is an IL-2 receptor found on the cell membrane. This receptor is released together with IL-2 by T cells. This suggests that sIL-2R is associated with the host immune status and severity in patients with active TB. The sIL-2R may be used as a marker to monitor TB patients on treatment.[30]

The difference in results in this study compared to previous studies is most likely because there is only 1 subject out of 39 total subjects who did not experience conversion. Reanalysis of a larger number of non-conversion groups was required to confirm the actual change of IL-2 values.

There was a weak and not-significant correlation between the IL-2 value and the AFB sputum smear ($0.2<r<0.4$; $P>0.05$). A positive R-value indicates that the higher the IL-2 value, the greater amount of AFB can be found in the smear. This result is similar to a study by Luo et al which showed no significant correlation between levels of IL-1β, sIL-2R, IL-6, and TNF-α in patients with lung TB, with the sputum smear classified as 1+, 2+, and 3+. Another study examined levels of IL-2, IL-7, IL-15, and IL-21 cytokines and also showed similar results. There were no significant differences in cytokine values between patients with bilateral and unilateral lung TB. There was also no significant cytokine levels difference in patients with and without cavities. Furthermore, there was no correlation between levels of IL-2, IL-7, IL-15, and IL-21 cytokines and the degree of AFB sputum smear in patients with lung TB. The absence of correlation between IL-2 values and the disease severity and bacterial load does not necessarily confirm their role in the course of TB disease. The IL-2 values are considered to have more influence on susceptibility or patient resistance to infection and do not reflect the pathogenesis of the disease too much.[31]

CONCLUSION

There was a significant decrease of IL-2 levels before treatment compared to 30 days after treatment, however there were significant differences in IL-2 levels before and 30 days after treatment between the conversion and non-conversion groups. There was a weak and not-significant correlation between IL-2 value and AFB sputum smear. A positive value of r indicates that the higher the IL-2 value, the greater the amount of AFB found in the smear results. The significant

reduction in IL-2 levels after 30 days of treatment also suggests its potential as a tool for evaluation and monitoring of therapy.

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