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Original Research Article

Factors associated with sputum culture conversion in patients with pulmonary tuberculosis

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ABSTRACT

Objective: The aim of this study was to determine what factors are associated with sputum culture conversion after 1 month of tuberculosis (TB) treatment.
Materials and methods: A total of 52 patients with new drug susceptible pulmonary TB were included in the study. Patients completed St. George respiratory questionnaire (SGRQ), they were asked about smoking, alcohol use, living conditions and education. Body mass index (BMI) measurements, laboratory tests (C reactive protein [CRP], vitamin D, albumin) were performed, and chest X-ray was done. After 1 month of treatment sputum culture was repeated.
Results: Culture conversion after 1 month of treatment was found in 38.5% cases. None of investigated social factors appeared to have an effect on conversion, but worse overall health status (as reported in SGRQ) and longer duration of tobacco smoking were detected in the ''no conversion'' group. Concentrations of albumin, CRP, X-ray score and the time it took *Mycobacterium tuberculosis* culture to grow also differed. Patients who scored 30 or more on SGRQ were more than 7 times as likely to have no conversion. However, the most important factor predicting sputum culture conversion was sputum smear grade at the beginning of treatment: patients with grade of 2+ or more had more than 20-fold higher relative risk for no conversion. Using receiver operating characteristic curve analysis, we also developed a risk score for no conversion.
Conclusions: The most important factors in predicting sputum culture conversion after 1 month of treatment were grades of acid-fast bacilli in sputum smears at time of diagnosis and scores of SGRQ.

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1. Introduction

According to the European Centre for Disease Prevention and Control (ECDC), Lithuania is still a high tuberculosis (TB) priority country [1] and, although the prevalence of TB is slowly decreasing, in 2015 there were still 41.89 new TB cases per 100 000 population [2]. Among those 11.5% were multi drug-resistant TB (MDR-TB) [2]. The prevalence of human immunodeficiency virus (HIV) infection in Lithuania was 4.8 per 100 000 population (2014) [3] and the main AIDS-defining illness was TB [4].

It is well known that TB is not only a medical condition but also a social problem. Patients diagnosed with TB are contagious and, at least at the beginning of treatment, are kept in hospitals where they are separated from their family, work and social life. It is a national policy to keep patients isolated until they become culture negative.

In the national guidelines [5] it is recommended that sputum smears and culture should be repeated after two months of treatment. Sputum smear conversion at this time is believed to be one of the best biomarkers for prognosis of treatment success without the probability of relapse in the future [6–8]. However, two months of isolation in hospital can make a significant impact on professional or personal life of a TB patient.

According to the previously published studies, the presence of cavitary TB disease appears to be associated with an increased time to sputum smear conversion [9]. It is assumed that cavitation is related to a higher grade of bacteria in the lungs. Other studies also found different factors that are associated with sputum culture conversion. It was shown that culture conversion could be influenced by history of smoking [10], body mass index (BMI) [11], sputum smear grade before the treatment [12], vitamin D concentration [13], albumin concentration [14] and various social factors [15].

In this study we performed the microbiological testing after 1 month of treatment and aimed to determine what factors were associated with faster sputum culture conversion in cases of pulmonary TB.

2. Materials and methods

The study was carried out in one of the largest TB hospitals in Lithuania. Approximately 95% of patients treated here are from Kaunas district, which is the second largest district in Lithuania and constitutes around 12.4% of Lithuania's territory and 20% of population. Only adults are treated in our hospital and directly observed treatment (DOT) is fully implemented as the staff ensures that patients are taking their medication seven days a week.

From November 2015 all patients with first-time diagnosis of pulmonary TB, meeting no exclusion criteria (significant morbidity due to other illnesses (e.g. cancer, autoimmune diseases, renal insufficiency); HIV positive; pregnant or breastfeeding) were asked to participate in this clinical trial. We selected these exclusion criteria to avoid the variability of laboratory tests and radiological changes, caused or influenced by other diseases, except TB. There were no patients with diabetes mellitus in our study group. During the period of one year (up to November 2016) 96 patients were included in the study, and 52 of them with drug susceptible pulmonary TB were analyzed in this article. Upon inclusion, acid-fast bacilli (AFB) had to be found in sputum with *Ziehl–Nielsen* histochemical reaction or positive *Mycobacterium tuberculosis* culture in MGIT BACTEC had to be detected. Selection process can be seen in Fig. 1. Two of sputum smear positive patients later were confirmed to have atypical mycobacteriosis and were excluded from the study. All patients in this study, eventually were bacteriologically confirmed to be infected with *M. tuberculosis* on MGIT BACTEC culture.

Quality control of smear microscopy was performed by the Central Tuberculosis Reference Laboratory. AFB smear positive results were as per World Health Organization/International Union Against Tuberculosis and Lung Disease grading: ''scanty'' with of 1–9 AFB per 100 oil immersion fields; ''1+'' with 10–99 AFB per 100 oil immersion fields; ''2+'' with 1–10 AFB per 1 oil immersion field and ''3+'' with >10 AFB per oil immersion field. All TB patients were offered rapid HIV testing foreseen in the National guidelines [3,5]. None declined to be tested. If a patient was found to be HIV positive, he/she was excluded from the trial.

Before the initiation of the TB treatment postero-anterior chest X-ray was done, patients completed St. George respiratory questionnaire (SGRQ) and answered the question about their overall health status (multiple-choice question with 5 possible answers: ''very good'', ''good'', ''average'', ''bad'', and ''very bad'') in the Lithuanian (native) language, they were asked about smoking habits, alcohol use, occupation, living conditions (rural or urban residence; family status) and education. The result of SGRQ was calculated using a program provided by St George's University of London. Body mass index (BMI) measurement, and laboratory tests from peripheral blood (C reactive protein (CRP), vitamin D, and albumin) were performed before the start of TB treatment. Pack-years of cigarette smoking were calculated based on the interview with a patient (by multiplying the number of packs of cigarettes smoked per day by the number of years the person has been smoking). Chest X-ray was evaluated by an experienced radiologist and the score of disease spread was calculated according to the method described by Ralph et al.: presence of baseline cavitation and the total percentage of lung affected were recorded and a score was calculated (CXR score = proportion of total lung affected (%) + 40 if cavitation is present) [16]. CRP, albumin and vitamin D testing was performed at the hospital of Lithuanian University of Health Sciences Kaunas Clinics, an externally quality-assured laboratory. We defined vitamin D values 70–250 nmol/L as optimal; 51–69 nmol/L, as insufficient, and below 50 nmol/L, as deficient. Reference values for CRP were 0–7.5 mg/L and for albumin, 35–48 g/L.

After 1 month of in-hospital DOT, sputum microscopy, sputum culture and CRP were repeated.

Statistical analysis was performed using SPSS version 23.0 for Windows (Statistical Package for the Social Sciences, Chicago, IL, USA). The following descriptive statistics were reported: proportions with their 95% confidence intervals for dichotomous variables and medians with their interquartile ranges (IQR) for continuous variables. Categorical variables were evaluated using the Pearson χ^2 test. Comparisons of

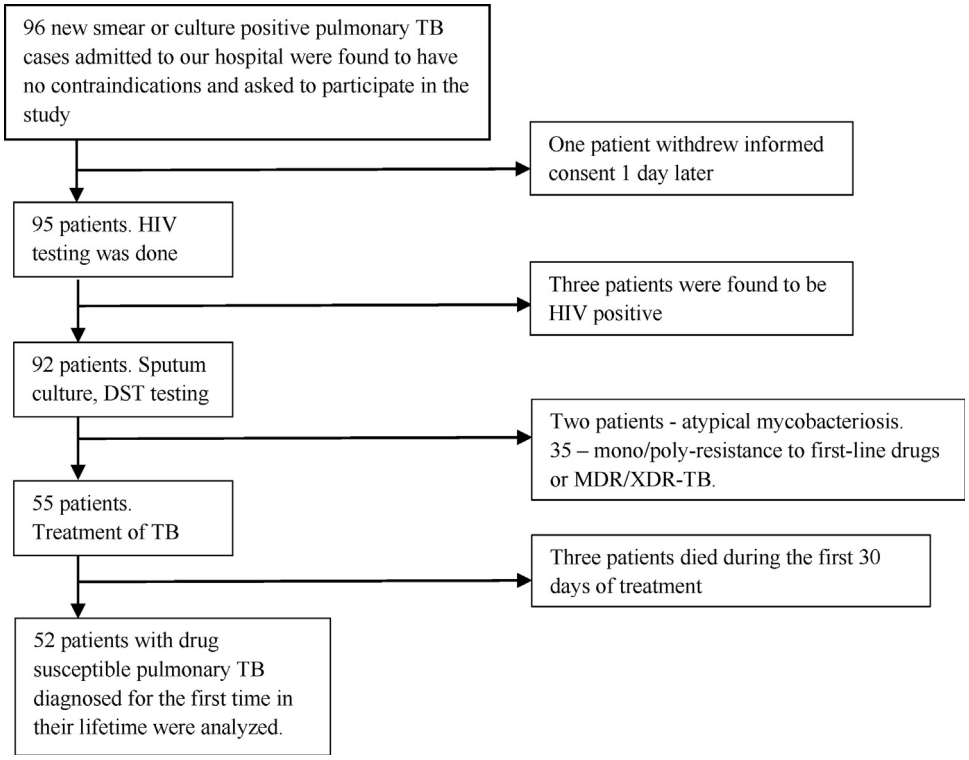

Fig. 1 – Flow chart of study participants. HIV, human immunodeficiency virus; DST, drug susceptibility testing; MDR-TB, multidrug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis.

continuous variables between ''conversion'' and ''no-conversion'' group were made using Mann–Whitney *U* test, as there were relatively few observations. $P \leq 0.05$ was considered statistically significant. Binary logistic regression was performed with dichotomous variables (continuous variables were transformed to categorical variables by splitting them into two groups with the median being a cut-off value). Dichotomous variables that had $P < 0.1$ were entered into the logistic regression model.

All patients were examined as part of a clinical research protocol approved by regional bioethics committee and informed written consent was obtained from all participants. All TB patients received the standardized TB treatment regimen in line with national TB treatment guideline [5] (isoniazid, rifampicin, ethambutol, pyrazinamide for 2 months, later continuing isoniazid and rifampicin for four months, with doses adjusted according to their weight).

3. Results

Culture conversion after 1 month of treatment was seen in 20 (38.5%) cases. Conversion after 1 month of treatment was confirmed by repeated sputum culture test after two months of treatment (at least 30 days apart).

Of the 52 patients, 40 (76.9%) were men. All were Lithuanian born, and there were no immigrants. None admitted to using any illicit drugs. Most had a place to live and only one was homeless. Other characteristics of the two groups can be seen in Table 1.

Two groups (those who converted, and those who did not) are relatively small, and we found no statistically significant difference of age, sex or BMI. BMI ranged from 16.56 to 34.05 kg/m^2, with 9 (15.4%) patients being overweight and 6 (11.5%) underweight (BMI <18.5). We did not detect any effect of BMI on sputum culture conversion. However, there were statistically significant differences in albumin concentrations (as seen in Table 1). Albumin concentration was significantly lower ($P < 0.05$) in patients, who did not convert at the end of 1 month of treatment. Vitamin D concentrations between two groups did not differ in a statistically significant way. Vitamin D levels ranged from 17.8 to 73.9 (mean, 43.387; SD, 2.01). Findings were abnormal in most of our patients: 51 patient (98.1%) had insufficient values of vitamin D. Out of those, 36 had deficient values (below 50 nmol/L). Only one patient had normal value of vitamin D before treatment initiation.

None of the investigated social factors (education, occupation, area of residence, family status or previous alcohol consumption) appeared to influence culture conversion. However, those with no conversion had higher scores on SGRQ ($P < 0.05$), and, in the same questionnaire, reported worse overall health status (Table 1). Patients with long history of tobacco smoking (20 pack-years or more) also converted less frequently ($P = 0.046$).

It was detected that concentration of CRP in blood plasma and CRX score were higher in the group with no sputum culture conversion ($P < 0.05$). Other finding that varied

Table 1 – Sociodemographic, clinical and laboratory characteristics by conversion status after 1 month of treatment.

Characteristics	Conversion group (n = 20)	No-conversion group (n = 32)	P value
Sex			0.677[b]
Male	16 (40.0)	24 (60.0)	
Female	4 (33.3)	8 (66.7)	
Age, median (IQR), years	36 (29–45)	37 (32.6–46.5)	0.587[a]
Age, years			1.0[b]
≤45	10 (38.5)	16 (61.5)	
>45	10 (38.5)	16 (61.5)	
BMI, kg/m^2, median (IQR)	22.37 (19.89–24.06)	22.21 (19.94–23.24)	0.837[a]
BMI, kg/m^2			0.895[b]
≤22	9 (37.5)	15 (62.5)	
>22	11 (39.3)	17 (60.7)	
Area of residence			0.070[b]
Urban	12 (52.5)	11 (47.6)	
Rural	8 (27.6)	21 (72.4)	
Family status			0.930[b]
Single	11 (37.9)	18 (62.1)	
With family/partner	9 (39.1)	14 (60.9)	
Occupation			0.508[b]
Employed	10 (43.5)	13 (56.5)	
Unemployed/retired	10 (34.5)	19 (65.5)	
Education			0.284[b]
High school or lower	10 (32.8)	21 (67.7)	
Higher education	10 (47.6)	11 (52.4)	
Health status (self-reported)			0.007[b]
Very good, good	11 (64.7)	6 (35.3)	
Average, bad, very bad	9 (25.7)	26 (74.3)	
SGRQ score, median (IQR)	22.13 (4.03–29.05)	32.79 (26.17–47.86)	0.009[a]
SGRQ score			0.008[b]
<30	15 (53.6)	13 (46.4)	
≥30	4 (17.4)	19 (82.6)	
Tobacco smoking history	15 (75)	28 (87.5)	0.246[b]
Pack-years of tobacco smoking, median (IQR)	12.5 (5–16)	20 (5–25)	0.330[a]
Pack-years of tobacco smoking			0.046[b]
<20	15 (50.0)	15 (50.0)	
≥20	5 (22.7)	17 (77.3)	
Alcohol consumption			0.963[b]
2–3 times/week or more often	7 (38.9)	11 (61.1)	
Less often	13 (38.2)	21 (61.8)	
Sputum smear grade			<0.0001[b]
3+	0	21 (65.6)	
2+	5 (25)	5 (15.6)	
1+	3 (15)	4 (12.5)	
Scanty	4 (20)	2 (6.3)	
0	8 (40)	0	
Sputum smear grade			<0.0001[b]
<2+	15 (71.4)	6 (28.6)	
≥2+	5 (16.1)	26 (83.9)	
CRP, median (IQR), mg/L	2.16 (1–7.8)	18.07 (5.57–41.32)	0.001[a]
CRP, mg/L			0.027[b]
<30	15 (51.7)	14 (48.3)	
≥30	5 (21.7)	18 (78.3)	
Albumin, median (IQR), g/L	36 (28–39)	25.5 (24–35)	0.012[a]
Albumin, g/L			0.046[b]
≤35	5 (22.7)	17 (77.3)	
>35	15 (50.0)	15 (50.0)	
Vitamin D, median (IQR), nmol/L	35.2 (24.5–43.5)	29.35 (24.58–38.55)	0.342[a]
Vitamin D, nmol/L			0.136[b]
<41	7 (28.0)	18 (72.0)	
≥41	13 (48.1)	14 (51.9)	
1st culture, median (IQR), days required to grow	14 (10–30)	9.5 (7–16.5)	0.015[a]
1st culture, days required to grow			0.031[b]
<10	4 (20.0)	16 (80.0)	
≥10	16 (50.0)	16 (50.0)	
CRX score, median (IQR)	53.33 (8.33–58.33)	95.83 (74.16–109.16)	<0.001[a]

Table 1 (*Continued*)

Characteristics	Conversion group ($n = 20$)	No-conversion group ($n = 32$)	*P* value
CRX score			<0.001[b]
<80	12 (70.6)	5 (29.4)	
≥80	3 (16.7)	15 (83.3)	

Values are number (percentage) unless noted otherwise.
[a] Mann–Whitney *U* test.
[b] Pearson chi-square test.
IQR, interquartile range; BMI, body mass index; SGRQ, St. George respiratory questionnaire; CRP, C reactive protein; CXR score, proportion of total lung affected (%) + 40 if cavitation is present.

Table 2 – Binary logistic regression analysis (stepwise selection) for factors associated with sputum culture conversion after 1 month of tuberculosis treatment (classification, 82.4% correct).

Factor	OR (95% CI)	*P* value
SGRQ total score (<30/≥30)	7.104 (1.262–39.992)	0.026
Tobacco smoking (<20 pack-years/≥20 pack-years)	5.191 (0.926–29.102)	0.061
Sputum smear grade before treatment (<2+/≥2+)	20.808 (3.774–114.717)	<0.0001

OR, odds ratio; CI, confidence interval; SGRQ, St. George respiratory questionnaire.

significantly between the two groups was the number of days it took the culture to grow – in the conversion group it took longer time period for *M. tuberculosis* to grow in liquid medium.

As seen in Table 2, the most important factor determining conversion after 1 month was the sputum smear grade before the start of treatment. As presented in Table 1, none of the patients, having smear grade of 3+ converted after 1 month of treatment, and those who had grade of 2+ or more were 20.808 times as likely not to convert after 1 month ($P < 0.001$) (Table 2). The score of SGRQ can also be used in the model predicting sputum culture conversion – patients who scored 30 or more on SGRQ before the start of treatment were 7.104 times as likely not to convert after the first month of treatment ($P = 0.026$). Tobacco smoking for 20 or more pack-years was also included in the regression model (Table 2); nonetheless, this factor did not appear to be statistically significant ($P = 0.061$).

We also tried to develop a no conversion risk score using the factors found to have an influence on culture conversion after 1 month of treatment. We constructed this score based on odds ratios of logistic regression (Table 2): if the patient scored 30 or more points on SGRQ, 7 points were allocated to no conversion risk score, and if he/she scored less on SGRQ, 0 points; in the patient was smoking for 20 or more pack-years, 5 points on risk score, and if less, 0 points; if sputum smear grade before the treatment initiation was 2+ or more, the patient got 21 points on risk score, and if less, 0 points. The minimum and maximum number of points was 0 and 33, respectively. The ROC curve is presented in Fig. 2.

Using the ROC curve, a cut-off value of 16.5 could discriminate no conversion with a sensitivity of 81.3% and a specificity of 75% (positive predictive value 83.88%, negative predictive value 71.48%).

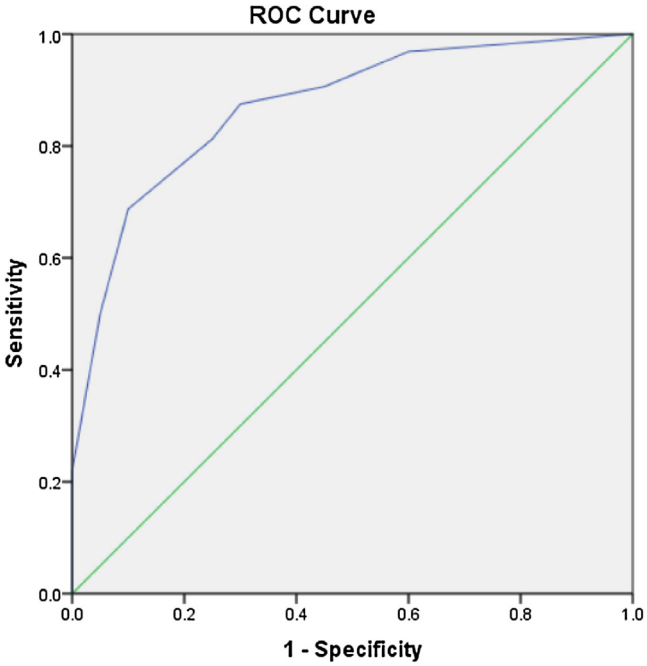

Fig. 2 – Receiver operating characteristic (ROC) curve analysis for sputum culture conversion, based on a developed score using SGRQ score, pack-years of smoking and sputum smear grade before the initiation of treatment. A ROC curve analysis was performed to investigate whether this score is able to predict the culture conversion. The corresponding area under the curve (AUC) value is 0.87 and the 95% confidence Interval (CI) is 0.773–0.968.

4. Discussion

We have identified that there are some factors which may contribute toward predicting sputum culture conversion event after 1 month of treatment, the most significant of them being sputum smear grade and SGRQ score before TB treatment initiation. The present study highlights that these findings can also have a practical application – there may be no need to repeat bacteriological testing in high smear grade patients after the first month of treatment, because of the unlikely event of sputum culture conversion. Our findings are comparable to those in the study of Mesquita et al. [17] that has shown a strong association between higher pre-treatment mycobacterial loads and higher risk for culture positivity at day 60 of treatment.

Another factor in predicting culture conversion was found to be a smoking history (smoking for more than 20 pack-years). It is known, that tobacco smoking is an important risk factor for TB [18]. Previous studies have also shown that tobacco smoking is associated with a considerably increased risk of advanced and more severe disease in the form of lung cavitations, positive sputum smear and culture results, and slower smear and culture conversion after initiation of treatment [18]. Other factors, such as CRP and albumin concentrations can also have a role in bacteriological outcome after 1 month of treatment. Previous studies have shown similar results: highest concentrations of CRP were found in patients with severe TB disease [19,20], lower albumin concentrations were associated with higher in-hospital mortality [21].

Practical application of vitamin D testing in TB patients is still debatable. There are conflicting evidence of vitamin D supplementation as an adjunct therapy of TB [22]. In the study of Tukvadze et al. a high-dose vitamin D3 regimen given to the patient with insufficient vitamin D concentrations safely corrected the deficiency, but did not improve the rate of Mycobacteria clearance from sputum in pulmonary TB cohort [23]. Similar results were demonstrated in other studies [24,25]. However, others have found that vitamin D significantly shortens time to culture conversion in patients with the *tt* genotype of the TaqI vitamin D receptor polymorphism [26] and serum concentrations of vitamin D were significantly lower in multidrug-resistant TB (MDR-TB) patients, furthermore they inversely correlated with time to sputum smear conversion [27]. Most of our patients had insufficient values of vitamin D. Although, vitamin D concentration was higher in the conversion group of our study, this difference was not statistically significant. It is stated that vitamin D deficiency might be a risk factor for TB development [28], however, similar tendencies of vitamin D deficiency are seen in our yet unpublished results with pneumonia patients.

In the current literature we were able to find only one validated chest X-ray score for pulmonary TB, which could be used in monitoring radiological changes in the lungs during the course treatment. In the study of Ralph et al. [16] this X-ray score was associated with baseline sputum smear grade. In patients with unfavorable outcomes this score was higher than in those with favorable outcomes. While using this score in our study, statistically significant differences were found in the two study groups, however more data are still needed to broaden our understanding in its practical use.

We found no studies evaluating quality of life questionnaires or SGRQ and its association with culture conversion. In our study statistically significant differences between conversion and no-conversion groups were shown, with lower scores of SGRQ and better overall health status more common in the conversion group.

Also, there were factors which did not appear to influence culture conversion. We found no difference of BMI between our study groups. There were previous studies on associations between BMI and TB mortality, but results of those were inconsistent [29,30]. Some studies found that lower BMI was associated with higher mortality among TB patients [31], one found no such association [29], and one – that overweight was associated with a lower mortality rate [32]. There are still few studies on BMI association with culture conversion, but most are done with MDR-TB patients [11].

Though previous studies state that excess alcohol use in TB patients is associated with greater mortality, lower rates of sputum culture conversion [33] and poor TB treatment outcomes [34], we found no statistically significant difference of alcohol consumption between conversion and no-conversion groups in our study.

We believe that one of the strengths of our study is it being prospective study of in-hospital patients on DOT. There is no doubt that patients were taking their medication and were not missing doses. All of them were in the same environment and were treated according to the same methodology. However, it cannot be assumed that the same results would be found if patients were taking medication in the community. All patients admitted to our hospital in the year the study was carried out were asked to participate, and study population can represent the population of TB patients in Kaunas district. One of the weaknesses of this study was relatively small enrollment numbers. However, it is still ongoing and more data will be available later.

5. Conclusions

According to the findings of our study, there are several factors that can be used in predicting culture conversion in pulmonary TB patients after 1 month of treatment. Most important of them were grades of acid-fast bacilli in sputum smears and scores of SGRQ.

Conflicts of interest

The authors state no conflict of interest.

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