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Adjunct Immunotherapy of Tuberculosis in Drug-Resistant TB and TB/HIV Co-Infected Patients

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ABSTRACT

Open-label, salvage anti-tuberculosis therapy (ATT) combined with DZHERELO (IMMUNOXEL), SVITANOK, and LIZORM – over-the-counter immunomodulators from medicinal plants – was conducted in 20 Ukrainian patients, comprising seven who had HIV co-infection. Except five patients with HIV, all other individuals had multidrug-resistant TB (MDR-TB) including 7 (35%) patients with XDR-TB. Patients hospitalized in our TB dispensary were treated under directly observed therapy (DOT) until repeated negative culture conversion and recuperation from radiological and clinical symptoms. The average duration of therapy was 16.2 ± 5.2 weeks (range 10.6-30.3; median 16). The mean time to bacterial clearance was 4.4 ± 1.8 weeks (range 1.3-8.9, median 4.3). All patients (95%), except one, gained weight, ranging between 3-17 kg with average 8.7 kg (P=0.000009). The liver function tests revealed that the level of total bilirubin had decreased from 15.5 to 11.6 μmol/L (P=0.009). Alanine transaminase (ALT) declined from elevated 53.1 IU/L to normal 30.4 IU/L level (P=0.001). Hemoglobin levels increased from 103.2 to 117.3 g/L (P=0.00005). Inflammation-associated, elevated leukocyte counts returned back to normal from 8.9 to 6.9 × 10^9 cells/L (P=0.003). Patients improved clinically and radiologically and were hence discharged from the hospital. These findings support prior trials indicating clinical benefit of adding immunomodulators to TB treatment regimens. The combination of ATT with botanical preparations enhances the clinical efficacy of DOT and is safe and beneficial even to patients with poor prognosis due to drug resistance and/or co-infection with HIV.

Keywords: AIDS, botanical, drug resistance, Ekomed, herbal, HIV, immunotherapy, MDR, Mycobacterium tuberculosis, phytoconcentrate, phytomedicine, phytotherapy, Ukraine, XDR

INTRODUCTION

The tuberculosis (TB) epidemic is on the rise in many countries, including Ukraine. If left untreated the active form of TB will kill two of every three people (Karachunskii 2000). This problem is further compounded by HIV co-infection, since one-third of AIDS-related deaths results from TB. Ukraine has the highest prevalence of TB/HIV co-infection in Eastern Europe (van der Werf *et al*. 2006). The effectiveness of TB therapy is significantly lower among patients with HIV/AIDS (Karachunskii 2000). The World Health Organization (WHO) estimates that a person with both HIV and TB infection is thirty times more likely to become ill with TB than a person with *Mycobacterium tuberculosis* infection alone (Reid *et al*. 2006). The rate of relapse and mortality are consistently higher even when TB/HIV patients are treated with anti-tuberculosis therapy (ATT) under directly observed treatment program (DOT) (Khauadamova *et al*. 2001). Drug resistance along with HIV-accompanying immunodeficiency is the main cause of treatment failure. Recently published survey of Nikolayevskyy *et al*. (2007) indicates that in Ukraine the multi-drug resistant form of TB (MDR-TB) was found in 27.3% of TB patients but was twice higher (54.8%) among formerly incarcerated individuals.

The first line of TB drugs includes isoniazid, rifampicin, ethambutol, pyrazinamide, and streptomycin. There are six classes of second-line TB drugs including fluoroquinolones: e.g., ciprofloxacin, moxifloxacin; aminoglycosides: e.g., amikacin, kanamycin; polypeptides: e.g., capreomycin, viomycin, enviomycin; thioamides: e.g. ethionamide, prothionamide; cycloserine; and para-aminosalicylic acid (Zaitzeva 2004). Other TB drugs, which are not on the WHO list, include: rifabutin; macrolides: e.g., clarithromycin; linezolid; thioacetazone; thioridazine; arginine; and vitamin D. MDR-TB is diagnosed when *M. tuberculosis* are resistant to at least isoniazid (H) and rifampicin (R), two most commonly-used, first-line drugs. The extensively resistant TB (XDR-TB), in addition to lack of sensitivity to H and R, is also resistant to any one of fluoroquinolones, and at least one of second-line injectable drugs, e.g., capreomycin, kanamycin, and amikacin (Migliori *et al*. 2007). This emerging form of TB caused worldwide concern after recently reported outbreak in Kwazulu Natal province of South Africa where 52 of 53 patients with XDR tuberculosis and HIV co-infection had died within 2 weeks from the time of diagnosis (Gandhi *et al*. 2006).

Immunomodulators DZHERELO, SVITANOK and LIZORM are made from a proprietary combination of medicinal plants and are commonly used in Ukraine for the management of TB and HIV infections, including patients with dual infection (Arjanova *et al*. 2006; Prihoda *et al*. 2006; Zaitzeva 2006; Chkhetiany *et al*. 2007; Nikolaeva *et al*. 2008). They have been approved in 1997 by the Ministry of Health of Ukraine as functional supplements with therapeutic indications. In 1999 DZHERELO and SVITANOK combination were specifically recommended as an immune adjunct to the therapy of pulmonary tuberculosis (Melnik *et al*. 1999). So far, the phytoconcentrates we have used in this study have been taken by several hundred thousand individuals for various indications including chronic bacterial and viral infections such as TB and HIV, autoimmune diseases,

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and malignancy (Chkhetiany *et al*. 2007).

Published studies have demonstrated that DZHERELO can significantly shorten the duration of treatment and helps to achieve higher response rate even in those who are HIV co-infected or have MDR forms of TB (Arjanova *et al*. 2006; Prihoda *et al*. 2006; Chkhetiany *et al*. 2007). DZHERELO has also been found to decrease the hepatotoxicity associated with ATT (Zaitzeva 2006). SVITANOK is commonly used for counteracting liver-damaging effect of chemotherapy and in hepatitis therapy. LIZORM is routinely used for alleviating symptoms of autoimmune disorders (Bodnar *et al*. 2002). Our study was aimed at evaluating the combined effect of DZHERELO, SVITANOK, and LIZORM in a representative sample of hospitalized patients who received the anti-TB therapy under DOT. Patients were selected among those who had particularly poor prognosis due to drug resistance and/or HIV co-infection.

MATERIALS AND METHODS

Patients

Twenty patients with active TB and poor prognosis due to resistant TB and/or HIV co-infection were selected to be given in addition to standard ATT the over-the-counter, immunomodulating phytopreparations manufactured by Ekomed company. The age of patients ranged between 24 and 58 years with mean/median age of 39.7/39.5 years. The female/male ratio was 4/16. Eleven patients presented with first-diagnosed TB and nine patients had previously treated, relapsed, i.e., chronic TB. Fifteen patients had drug-resistant TB, including seven with XDR-TB, and five patients in TB/HIV subgroup had drug-sensitive TB. All study patients presented with acute symptoms of pulmonary TB that required hospitalization. Most common symptoms were prolonged heavy cough, pain in the chest, high fever, profuse night sweats, fatigue, and loss of weight and appetite. Active pulmonary tuberculosis was certified by a medical history and clinical findings compatible with tuberculosis, a chest X-ray showing lung involvement, and positive sputum smear for acid-fast bacilli (AFB) and the culture of *M. tuberculosis*. The diagnosis of HIV infection was established by standard ELISA test further confirmed by Western blot. All HIV-positive patients were in advanced stage III of HIV infection. None of the patients received anti-retroviral therapy prior to and during follow-up. The conduct of the study was approved by the internal review board (IRB) of Lisichansk TB dispensary. The participation in this study was voluntary and patients were eligible to enroll only after signing the written consent.

Treatment regimen

Individualized, first- and second-line anti-TB drugs were administered to hospitalized patients based on physician's decision prior to or after results of drug susceptibility tests. Drugs were administered under DOT schedule. In addition to ATT, patients received a daily dose of DZHERELO which was given as 30 drops diluted in a half-glass of water 30 minutes before breakfast. Some patients received DZHERELO-PI – slightly modified form of DZHERELO. The same dose, 30 drops, of LIZORM and SVITANOK were given before lunch and supper respectively. Sputum smear and culture examinations for AFB were performed at monthly intervals. The decision to discharge was based on at least twice-repeated negative culture outcome and satisfactory clinical and radiological findings.

Anti-tuberculosis drugs and phytopreparations

All anti-TB drugs were procured through the centralized national supply system of Ukraine. The over-the-counter phytoconcentrates, DZHERELO, SVITANOK, and LIZORM, were generously supplied by Ekomed company. DZHERELO (IMMUNOXEL) contains concentrated aqueous-alcohol extract from medicinal plants such as aloe (*Aloe arborescens*), common knotgrass (*Polygonum aviculare*), yarrow (*Achillea millefolium*), purple coneflower (*Echinacea purpurea*), centaury (*Centaurium erythraea*), snowball tree berries (*Viburnum opulus*), nettle (*Urtica dioica*), dandelion (*Taraxacum officinale*), sweet-sedge (*Acorus calamus*), oregano (*Oreganum majorana*), marigold (*Calendula officinalis*), seabuckthorn berries (*Hippophae rhamnoides*), elecampane (*Inula helenium*), tormentil (*Potentilla erecta*), greater plantain (*Plantago major*), wormwood (*Artemisia* sp.), Siberian golden root (*Rhodiola rosea*), cudweed (*Gnaphalium uliginosum*), licorice (*Glycyrrhiza glabra*), fennel (*Foeniculum vulgare*), chaga (*Inonotus obliquus*), thyme (*Thymus vulgaris*), three-lobe beggarticks (*Bidens tripartite*), sage (*Salvia officinalis*), dog rose (*Rosa canina*), and juniper berries (*Juniperus communis*). SVITANOK contains flowers of immortelle (*Helichrysi arenarii*), barberry roots (*Berberis vulgaris*), chicory roots (*Cichorium intybus*), coriander seeds (*Coriandrum sativum*), marigold (*Calendula officinalis*), wormwood, and maize cores with stigmas (*Zea mays*). LIZORM contains concentrated aqueous-alcohol extract from barberry roots (*Berberis vulgaris*), aronia berries (*Aronia melanocarpa*), St. John's Wort (*Hypericum perforatum*), centaury, nettle, common knotgrass, wild strawberry leaves (*Fragaria vesca*), greater celandine (*Chelidonium majus*), and immortelle. All phytopreparations were approved in 1997 by the Ministry of Health of Ukraine as dietary supplements. In 2006 they received the status of a functional food – superior category of herbal supplements which can carry medical claims substantiated by clinical evidence.

TB drug resistance testing

The drug resistance profile to first- and second-line TB drugs was tested with a commercially supplied kit (Tulip Diagnostics, Goa, India). The cultures of *M. tuberculosis* derived from sputum of each patient were inoculated into ready-to-use tubes containing TB drugs incorporated at manufacturer-predetermined concentrations into standard Löwenstein-Jensen agar slants. The inoculae were incubated at 37°C and checked periodically until appearance of colonies in control tubes without drugs. The calculation of the proportion of resistant bacilli was done by comparing counts on drug free and drug-containing Löwenstein-Jensen medium, essentially as described by Laszlo *et al*. (1997).

Statistical analysis

The obtained results were analyzed with the aid of statistical software STATMOST (Datamost, South Sandy, UT). All statistical analyses were done on intent-to-treat basis, involving the total number of patients without subgrouping them into responders and non-responders. Simple statistical calculations such as determination of standard deviation, mean and median, were performed with the same software. Where available the baseline values relative to the end of study values were evaluated by paired or unpaired Student t-test. When required the stratification analysis of patients was conducted to reveal the difference between distinct clinical categories. The probability values were considered as significant at $P \leq 0.05$ cut-off value.

RESULTS

The duration of DOT ranged between 10.6-30.3 weeks with average/median 16.2/16 weeks (**Table 1A, 1B**). The treatment lasted until patients were discharged from the dispensary upon twice-repeated negative culture findings and clinical and radiological improvements. The time to negative culture conversion ranged between 9-62 days with mean/median 30.6/30 days. Mycobacterial clearance was confirmed by repeated cultures at monthly intervals.

There was no difference between chronic, previously treated TB versus first-diagnosed TB cases in terms of days to discharge, i.e., 111.6 vs. 114.8 (P=0.42) or days to mycobacterial clearance, 33.7 vs. 28 (P=0.16). A similar stratification analysis comparing TB/HIV with TB alone patients reveals that patients with dual infection appear to require longer treatment, i.e., 127.9 vs. 105.5 days, but the difference was not statistically significant (P=0.15). Similarly, negative culture conversion occurred about nine days later in TB/HIV individuals than in TB patients, i.e., 36.1 vs. 27.5 days, but the difference was not statistically reliable (P=0.08). The comparison of treatment outcomes between

Table 1A Baseline and outcome characteristics of TB patients treated with ATT in combination with Dzherelo, Svitanok, and Lizorm.

No.	Sex	Age	TB infection	TB drug resistance*	HIV status/AIDS stage	Days on DOT	Days to negative culture
1/54	M	30	Primary	MDR; H/R/Z/O	-	74	10
2/57	M	58	Primary	MDR; R/ETH/CPX/PFX	-	122	30
3/73	M	38	Chronic	MDR; S/H/ETH/PAS	-	131	30
4/78	F	40	Primary	MDR; H/R/K/PAS/Prothio	-	77	9
5/92	M	32	Primary	MDR; H/E/PAS/RFB	-	122	55
6/492	M	47	Chronic	MDR; H/R/E/K	-	75	28
7/56	M	42	Primary	XDR; H/R/E/K/O/PAS	-	74	23
8/64	M	47	Primary	XDR; H/R/S/K/L/PFX	-	133	22
9/68	M	52	Primary	XDR; H/R/A/PFX/PAS	-	117	37
10/84	M	44	Primary	XDR; H/R/ETH/K/L/PAS	-	143	34
11/156	M	25	Chronic	XDR; H/R/Z/K/O/A/PAS	-	89	25
12/532	M	48	Chronic	XDR; H/R/K/A/O/PAS	-	122	35
13/627	M	35	Primary	XDR; H/R/K/A/CPX/PFX	-	93	20
14/59	M	47	Primary	-	+/III	212	34
15/61	M	39	Chronic	-	+/III	107	38
16/161	F	34	Chronic	-	+/III	98	34
17/185	F	24	Chronic	-	+/III	74	24
18/295	M	45	Chronic	-	+/III	183	27
19/72	M	27	Chronic	MDR; H/K/A/PAS	+/III	125	62
20/481	F	39	Primary	MDR; H/R/K/Prothio	+/III	96	34
20	4/16	39.7 ± 9.2	9/11	5/15	7/13	113.4 ± 36.7	30.6 ± 12.5

*Criteria for definition of XDR are as per WHO recommendation. ATT drugs are abbreviated as follows: Isoniazid (H), Rimfapicin (R), Pyrazinamide (Z), Ethambutol (E), Streptomycin (S), Levofloxacin (L), Ofloxacin (O), Ciprofloxacin (CPX), Pefloxacin (PFX), Kanamycin (K), Amikacin (A), Para-aminosalicylic acid (PAS), Rifabutin (RFB), Ethionamide (ETH), Prothionamide (Prothio)

Table 1B Baseline and outcome characteristics of TB patients treated with ATT in combination with Dzherelo, Svitanok, and Lizorm.

No.	Sex	Age	Leukocyte × 10^9 L		Hb g/L		Weight change kg		Total bilirubin μmol/L		ALT IU/L	
			before	after	before	after	before	after	before	after	before	after
1/54	M	30	9.4	12	82	104	60	68	32.4	11.7	37	50
2/57	M	58	9.8	6	120	123	66	78	14	10.5	37	50
3/73	M	38	14	6	108	120	52	68	16.3	10.5	62	12
4/78	F	40	10	5.2	100	116	52	56	14	10.5	25	12
5/92	M	32	5.8	6	110	122	75	85	10.5	10.7	50	12
6/492	M	47	8	6.8	106	122	66	78	14	19.7	75	50
7/56	M	42	11.6	8.1	122	114	59	68	10.5	11.7	25	50
8/64	M	47	9.1	9.1	108	116	52	62	11.7	10.7	62	12
9/68	M	52	11	6	100	118	64	74	11.7	10.4	75	12
10/84	M	44	4.5	6.8	120	118	63	69	18.6	10.5	12	50
11/156	M	25	8.2	6	109	120	65	78	10.5	10.5	62	12
12/532	M	48	8.8	5.3	88	122	72	78	14	10.5	37	12
13/627	M	35	9	10	88	118	50	63	32.4	10.5	25	12
14/59	M	47	6.8	6.9	128	118	77	80	11.7	10.5	50	50
15/61	M	39	6.5	4.9	106	112	65	76	10.5	11.5	75	50
16/161	F	34	5.4	5.2	105	118	58	67	20.9	10.5	42	12
17/185	F	24	6.5	7.3	95	118	63	70	10.5	10.5	75	50
18/295	M	45	9	6.8	94	120	61	68	18.6	18.6	112	50
19/72	M	27	11	6.2	102	116	76	66	16.4	10.5	37	12
20/481	F	39	13.3	7.1	72	110	43	60	11.7	10.5	87	37
20	4/16	39.7 ± 9.2	8.9 ± 2.5	6.9 ± 1.8	103.2 ± 14	117.3 ± 4.6	61.9 ± 9.1	70.6 ± 7.5	15.5 ± 6.5	11.6± 2.6	53.1 ± 27	30.4 ± 17.5
			Mean decrease = 2 × 10^9 L P=0.003		Mean gain = 14.1 g/L P=0.00005		Mean gain = 8.7 kg P=0.000009		Mean decrease = 3.9 μmol/L P=0.009		Mean decrease = 22.7 IU/L P=0.001	

15 drug-resistant and 5 drug-sensitive cases also failed to reveal statistical difference. Time to negative culture was 30.3 vs. 31.4 days and time to discharge 106.2 vs. 134.8 days with probability values P=0.4 and P=0.18, respectively.

At the end of study almost every patient had gained substantial lean body mass – an effect that was evident within one month from initiation of the therapy. Except one TB/HIV patient (#19) who lost 10 kg, all other patients gained weight, ranging between 3 and 17 kg. The average accrual in lean body mass was 8.7 kg (median 9.5 kg), which was statistically highly significant as evidenced by a paired Student's *t*-test (P=0.0000009).

The potential hepatotoxicity of ATT combination with herbal preparations was monitored by quantitative liver function tests. Surprisingly, despite intensive chemotherapy patients have shown signs of better liver function. The level of total bilirubin had decreased from mean 15.5 to 11.6 μmol/L – a favorable change that was statistically significant (P=0.009). Similarly, the values of alanine transaminase (ALT), another marker of liver damage, have declined from abnormally high (53.1 IU/L) to normal levels (30.4 IU/L) – a change that was also statistically significant (P=0.01).

Another phenomenon observed during therapy is a reversal of baseline anemic state and pro-inflammatory condition – symptoms very common in TB and HIV. Most patients at study entry displayed signs of anemia and had abnormally elevated leukocyte counts. At the end of treatment these parameters were improved in a statistically significant manner. The levels of hemoglobin had risen from 103.2 to 117.3 g/L (P=0.00005), whereas leukocyte counts returned back to normal levels from 8.9 to 6.9 × 10^9 cells/L (P=0.003).

Flow cytometry measurements of T lymphocyte counts conducted at study entry and at the end of follow-up were

Table 2 Changes in absolute CD4+ and CD8+ T-lymphocyte counts among TB/HIV patients

No.*	Sex	Age	CD4+ cells		CD8+ cells		Ratio of CD4/CD8 cells	
			Before	After	Before	After	Before	After
14	M	47	449	355	1260	1422	0.3563	0.2496
15	M	39	336	668	1128	824	0.2979	0.8107
19	M	27	412	715	501	515	0.8224	1.3884
16	F	34	458	746	504	510	0.9088	1.4628
17	F	24	372	204	1087	808	0.3422	0.2525
18	M	45	200	705	1607	764	0.1245	0.9228
Mean±SD			371.2 ± 95.7	565.5 ± 228	1014 ± 436.8	807 ± 332.8	0.4753 ± 0.315	0.8478 ± 0.527
P=by paired Student's *t*-test			P=0.066		P=0.11		P=0.03	

* The sequence of patients' numbers corresponds to that shown in **Table 1**.

available in 6 of 7 TB/HIV patients (**Table 2**). The helper CD4+ cells declined in two patients, while remaining patients had displayed an increase in their lymphocyte numbers. From average 371 cells/μl at baseline they have risen to 566 cells/μl – an increase equal to 52% (P=0.07). The absolute numbers of CD8+ T-lymphocytes appeared to decline but no statistical significance has been reached (P=0.1). The increase in CD4 cells and decline in CD8 cells resulted in almost doubled ratio of CD4/CD8 cells, i.e., from baseline 0.475 to 0.848 at the end of study (P=0.03).

DISCUSSION

Tuberculosis remains an enormous global health problem. There are 8-9 million new cases and 1.5-2 million deaths from TB annually. Despite the overwhelming burden of disease, no new compounds were developed in last 40 years and current strains of TB are becoming resistant to existing drugs. The emergence of XDR-TB raises a serious concern of epidemic of virtually untreatable TB. It is clear that currently available chemotherapies for the treatment of TB are not perfect – they require multiple tuberculosis drugs to be taken for long periods of time. The duration of therapy, coupled with the side effects, often results in poor patient adherence, treatment failure, and the emergence of drug resistance (Zaitzeva 2004). The results of this small-scale study, consisting of representative group of drug-resistant patients from our dispensary, indicate that when tuberculosis drugs are combined with immunomodulating herbal preparations, DZHERELO, SVITANOK, and LIZORM, they are able to produce rapid clinical and radiological improvements and disappearance of *M. tuberculosis* from a sputum culture within one month from treatment initiation.

Our findings support prior clinical studies of ATT that were conducted mostly with DZHERELO and occasionally with DZHERELO and SVITANOK combination (Melnik *et al*. 1999; Arjanova *et al*. 2006; Prihoda *et al*. 2006; Zaitzeva 2006; Chkhetiany *et al*. 2007). Our results indicate that the combination of three phytoconcentrates is even more effective in enhancing the efficacy of ATT and reducing the duration of treatment. Conversion of sputum mycobacterial culture from positive to negative is considered the critical interim indicator of the efficacy of anti-TB intervention (Holtz *et al*. 2006). We observed culture conversion at median 32 days (range 10-62 days). This is twice shorter than reported median 60 days culture conversion time (range 4-462 days) among drug-resistant TB patients in Latvia who were treated with first- and second-line TB drugs (Holtz *et al*. 2006). Same time to culture conversion, i.e., 2.1 months, was reported by Yew *et al*. (2003) in their retrospective MDR-TB study in Hong Kong. However, their mean duration of successful chemotherapy was 14.5 months as opposed to 3.9 months in our study. Similar range of therapy duration for drug-resistant TB, i.e., 11-24 months, was reported by Japanese investigators (Yoshiyama *et al*. 2007). If these studies are representative of best success rates in MDR-TB therapy then our immunomodulatory intervention affords twofold reduction in culture conversion time and shortens treatment duration by at least three-fold.

Numerous published studies have shown that patients with dual infection are less susceptible to TB treatment and had very poor prognosis despite best available standard of care (Karachunskii 2000; Khauadamova *et al*. 2001; Dean *et al*. 2002; Reid *et al*. 2006). Our patients with TB/HIV appear to require more time to achieve culture conversion and were prone to remain in the hospital longer than individuals without co-infection. However, due to limited sampling we were not able to confirm this impression by statistical analysis. We would need larger cohort of patients to determine whether TB/HIV patients are more refractory to the immunotherapy. Paradoxically, we did not see any difference in duration of treatment between TB patients with primary and chronic infections. Similarly, no meaningful pattern appeared when we compared drug-sensitive and drug-resistant patients for difference in time to culture conversion and duration of DOT. At least, theoretically, those with previously failed treatment or MDR-TB would require longer time to clear the infection (Sacks *et al*. 2001). These observations suggest that, unlike ATT, our phytoconcentrates do not have direct effect on *M. tuberculosis* growth and appear to act through modulation of the immune response (Melnik *et al*. 1999; Pylypchuk 2003).

No adverse effects attributable to the use of phytoconcentrates were observed at any time during therapy. Contrary, as a result of combination treatment our patients enjoyed better quality of life and were tolerating ATT much better than those who received ATT without phytotherapy. Despite liver damaging ATT regimen, our patients had shown amelioration of the liver function as evidenced by normalization of ALT and bilirubin. This illustrates that herbal supplementation with DZHERELO, SVITANOK, and LIZORM is safe and can neutralize or even reverse the hepatotoxicity of anti-tuberculosis drugs. The levels of hemoglobin improved markedly indicating that anemia was no longer of concern to our patients. This finding is highly relevant to the fact that anemia is an independent factor associated with an increased risk of mortality (O'Brien *et al*. 2005). TB-associated inflammation is another factor associated with poor prognosis (Feshchenko *et al*. 1997; Breen *et al*. 2004). Elevated leukocyte counts, indicative of ongoing inflammatory reaction, became normal as well. These beneficial effects were observed in all three categories of TB patients, i.e., MDR-TB, XDR-TB, and patients with HIV co-infection.

It is well know that the expansion of helper T-cell population and increased ratio of CD4/CD8 cells are associated with better prognosis in TB and HIV patients (Rodrigues *et al*. 2002). In TB/HIV group of patients we had 6 individuals out of 7 for whom we had data on such immune parameters. The measurement of CD4+ T-lymphocyte counts revealed that, except two individuals, four other patients had significantly higher number of cells at the end of follow-up. The ratio of CD4 to CD8 cells had almost doubled which was due to both an increase in CD4 and decrease in CD8 numbers (P=0.03). However, the P values for CD4 (P=0.07) and CD8 counts (P=0.1) were above ≤0.05 significance threshold. This may have been due to small sample size. In prior studies with larger number of patients the positive changes in absolute and relative T lymphocyte numbers as well as CD4/CD8 ratio were always highly significant (Chkhetiany

et al. 2007; Nikolaeva *et al*. 2008).

Tuberculosis is a wasting disease (Schwenk and Macallan 2000). Many patients with TB and/or HIV, especially in advanced disease stage, suffer from cachexia. This condition is poorly manageable and is one of the leading factors contributing to higher morbidity and mortality (Edwards *et al*. 1971; Villamor *et al*. 2006). Khan *et al*. (2006) reported that patients with underweight problem had higher risk of TB relapse and that changes in weight observed during early stages of treatment were an independent predictor of disease progression. The outstanding feature associated with our therapy is a dramatic body weight gain. The results show that 19 out of 20 (95%) patients had gained lean body mass equal to almost 13% of baseline weight (P=0.0002). TB drugs seldom enhance body weight (Paton *et al*. 2004). The only known to us report of significant weight gain in TB patients has been described by Donald *et al*. (1997). In their placebo-controlled study the increase in body mass, i.e., mean gain 8.9 kg, of magnitude similar to ours, has been described when TB patients were administered plant-originated β-sitosterol and sitosterolin. Unfortunately, these phytosterols had no effect on the rate of mycobacterial clearance. Other interventions which enhance weight but without effect on TB are corticosteroids and nutritional supplements (Smego and Ahmed 2003; Paton *et al*. 2004). The remarkable property of herbal immunomodulators DZHERELO, SVITANOK, and LIZORM in reversing weight loss along with substantiated adjunct effect on both TB and HIV will be particularly advantageous to those who live in resource-poor countries, where malnutrition is a common occurrence and deaths are more prevalent due to this cause (Farmer *et al*. 1991; Yew and Leung 2006).

Our study is unique since for the first time it reports treatment of Ukrainian patients with the XDR form of TB. Despite very poor prognosis we were able to achieve bacterial clearance within 4 weeks and attain major clinical and radiological improvements. As a result these patients were discharged from the dispensary after 110 (median 117) days. Our results contrast to two available clinical studies dealing with treatment of XDR-TB. In a report from South Africa 52 out of 53 (98%) patients had died within two weeks from diagnosis (Gandhi *et al*. 2006). However this extreme mortality rate might not be representative of the situation when more advanced clinical care is available. The retrospective study published by Kim *et al*. (2007) indicates that in South Korea the failure rate due to XDR-TB was 44.2%, whereas 27.4% patients with MDR did not respond to the therapy. Our experience is limited since we had only seven patients with XDR-TB and we need to confirm our findings in a larger cohort. According to published surveys on global prevalence of XDR-TB, the occurrence of XDR patients among MDR-TB cases is in 10-20% range (CDC 2006; Migliori *et al*. 2007; Shah *et al*. 2007). If these estimates are correct we will be able to find and recruit sufficiently large XDR population for an expanded study in the future.

It is agreed that immune-based therapies are urgently needed to complement TB drug discovery (Johnson *et al*. 2003; Kaufmann 2006; Achkar *et al*. 2007). We also believe that the immunotherapy must be the indispensable part of therapeutic interventions against tuberculosis (Pylypchuk 2003). Many potent immunomodulators are available against bacteria, protozoa, fungi and viruses (Ershov 2003). While often effective their mechanism is poorly understood in most cases. This drawback should be balanced against clinically confirmed benefits. From the review of available to us medical literature it is apparent that very few medicinal plants have shown scientifically confirmed TB-curing properties. Recently reported story, describing how the Zulu's traditional herbal medicine became a candidate TB drug, further highlights the difficulties of finding and implementing an effective TB remedy from botanical sources (Bladt and Wagner 2007). Nonetheless, some medicinal herbs were shown to modulate the immune response to TB (Tomioka 2004), while others exerted direct or indirect antimycobacterial activity (Klun and Youmans 1973; Newton *et al*. 2000). Unfortunately, we do not know which active ingredients in our multi-herb preparations are responsible for the observed clinical effect. It is unlikely that they act as tuberculostatic agents since *in vitro* growth of *M. tuberculosis* reference strains H32 and H37Rv was not affected directly either by DZHERELO or SVITANOK (Melnik *et al*. 1999) and diseases etiologically unrelated to TB were responsive to these preparations (Bodnar *et al*. 2002). It is clear that this issue must be further investigated by experts in medicinal plants.

Our study provides further evidence of safety and efficacy of DZHERELO, SVITANOK and LIZORM combination, former two of which were recommended in Ukraine as an immune adjunct to TB therapy (Melnik *et al*. 1999). In conclusion, we must emphasize that in this small study all patients were amenable to the therapy. The multi-country analysis of MDR-TB treatment outcomes has shown that cure rates were 52 and 29% for newly-diagnosed and retreated TB cases, respectively (Espinal *et al*. 2001). Thus, it is possible that when larger group of patients is evaluated then a certain number of treatment failures may emerge. Thus, additional studies need to be conducted in order to develop better understanding of this unique combination and to increase treatment options for TB patients with poor prognosis.

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