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ORIGINAL ARTICLE

Amputation, psychological consequences, and quality of life among Romanian patients

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Abstract

Amputation of the limb is a major psychological health issue that was not fully studied. Patients and their families struggle with symptoms of depression, posttraumatic stress disorder, anxiety, phantom limb phenomena, etc. There is also a tremendous impact on the person's quality of life.

Aim: This study aimed to assess the psychological symptoms of depression, anxiety, and the quality of life among persons suffering from amputation of the limb.

Methods: 31 respondents agreed to participate in the study. They were recruited from the Department of Orthopaedics and Traumatology, University Emergency Hospital, Bucharest, Romania. The medical conditions and the socio-demographic data (i.e., gender, marital status, income, etc.) along with the assessment of the psychological conditions and quality of life were contributions to the statistical analysis of the study.

Results: This study showed that levels of depression are moderate to severe for a significant percent of the population (i.e., a "moderate to severe" or "severe" level of depression was presented by 29% of the patients according to the PHQ-9 test, and 16.1% of the patients according to Beck's Depression Inventory). Moreover, the levels of anxiety were relatively higher than those of the general population (i.e., severe anxiety was confirmed by the GAD-7 test in the case of 38.7% of the patients) and for most of them the quality of life was impaired.

Discussion: Amputation occurs mainly in adults/ elderly aged over 50 years, married, living in urban areas, with different levels of depression and anxiety. At least half of them had a self-perceived health condition that was deteriorated, their physical and role functioning being primarily affected.

Conclusion: There is a need for psychological support of the patients suffering from limb amputation as most of the studies showed high prevalence of depression and anxiety. Socio-demographic factors also represent important elements in defining the quality of life of those patients.

Keywords: amputation, anxiety, depression, quality of life, rehabilitation

Introduction

Amputation of the limb represents a major event in a person's life with consequences that have not been fully studied. Physical consequences are mainly related to the inability to perform different life tasks, which were part of the person's life before. This creates a tremendous stress [1] that interferes with the person's life. There is a series of events and medical conditions that result in the amputation of the limb, like diabetes, peripheral vascular secondary infection, disease, tumor and psychiatric conditions. There are different types of amputation: foot, transtibial, through ankle, knee, transfemoral [2].

After the amputation, most of the patients express psychological problems like anxiety [3-5], depression [3-7], posttraumatic stress disorder and pain [8], etc., which affect their quality of life [9-11], along with their medical condition. There are patients who develop "mutilation anxiety" [1] and change their self-esteem due to changes suffered by their body image [12]. Moreover, 50%-80% of the amputee patients experience a phantom limb pain, while others develop residual limb pain, back pain, contralateral join pain [8].

Study design

A cross-sectional survey involving the of Orthopaedics Department and Traumatology of the University Emergency Hospital, Bucharest, Romania, was made. A cohort of 31 patients agreed to participate in study. This study obtained institutional review board approval from the **Ethics** Committee of the University Emergency Hospital, Bucharest.

Study setting and population

A set of self-report questionnaires designed to measure depression, anxiety, and the quality of life, was distributed.

Questionnaires were administered to 31 respondents under the direct supervision of a clinical psychologist, with the permission of their attending physician. Special attention was paid to the informed consent of each respondent in the study.

Instruments

EORTC QLQ-C₃o (3rdversion) is a self-report inventory, which consists of 30 items measuring the quality of life of clinical patients [13]. Items are scored on a four-point Likert scale, except for the 29th and the 30th items that are scored on a seven-point Likert scale. An example of using EORTC QLQ-C₃o was illustrated by King [14].

PHQ-9 (Patient Health Questionnaire-9) is a screening instrument, widely accepted for assessing the degree of depression severity in clinical patients [15]. The instrument has 9 items that are scored on a four-point Likert scale.

GAD-7 (General Anxiety Disorder-7) is also a screening instrument, widely accepted for the measurement of the severity of anxiety in clinical patients [16]. The instrument has 7 items that are scored on a four-point Likert scale.

Beck's Depression Inventory-Second Edition (BDI-II) - Romanian version is a self-report inventory that consists of 21 items designed to measure depression symptoms in adolescents and adults and represents a revised version of Beck's Depression Inventory [17]. Most of the items are scored on a four-point Likert scale, except for the 16th and the 18th items that are scored on a seven-point Likert scale.

Results

The profile of the study consisted of the 31 respondents who underwent an amputation surgical procedure through a set of relevant

socio-demographic data (see **Table 1**). The variable "age" was coded into six predefined categories, 25 patients (80.6%) being over 50 and 64.5% being males. Most patients were married (58.1%), one in four patients resided in rural area (25.8%). 29% of the patients had at most completed primary or secondary school, 61.3% of the patients had completed vocational school or high school, and only 9.7% had higher education.

Approximately one in three patients (35.5%) considered that their financial situation was below average, 54.8% evaluated their financial condition as average, and 9.7% considered that their financial situation was above average. Most patients were retired (61.3%) and one of them (3.2%) was unemployed; the remaining members of the target group worked in different fields of activity. One in two patients was a smoker (51.6%), 19.4% were former smokers and 29% were non-smokers. Most patients (54.8%) indicated that their relatives did not "ever" suggest to them that they drank too much, for

29% it was sometimes suggested that they drank "occasionally" and for 16.1% of them, their relatives often suggested them that they drank "too much".

One in two patients underwent a surgical amputation in the past six months, 12.9% within 6-12 months, 19.4% between one and two years and 16.1% over three years, starting from the moment the questionnaire was applied. Of all the patients, 71% underwent psychological counseling treatment. One in patients (32.3%) declared accommodated "easily" or "very easily" to the new body image, and one in three mentioned they accommodated "quite hard" or "hard" to the new image, while 35.5% declared they have found it very difficult to adapt to the new body image.

The medical diagnosis for the 31 patients varied quite widely, most being diagnosed with "Lower limb amputation" (i.e., nine cases), "Gangrene" (i.e., four cases) and "Lower limb obliterative arteriopathy" (i.e., four cases).

Table 1. Respondents who underwent an amputation surgical procedure through a set of relevant socio-demographic data

Age	Frequency	Percent	Have your relatives ever suggested that you drank too much?	Frequency	Percent
18-29	2	6.5	often	5	16.1
30 - 39	1	3.2	sometimes	9	29
40 - 49	3	9.7	never	17	54.8
50 -59	5	16.1	Total	31	100
60 - 69	13	41.9	How long ago was the amputation performed?	Frequency	Percent
70 - 79	7	22.6	Under six months	16	51.6
Total	31	100	Between 6 and 12 months	4	12.9
Gender	Frequency	Percent	Between 1 and 2 years	6	19.4
Male	20	64.5	Over three years	5	16.1
Female	11	35.5	Total	31	100
Total	31	100	Therapy followed by psychological counseling	Frequency	Percent
Marital status	Frequency	Percent	yes	22	71
Single	4	12.9	no	9	29
Married	18	58.1	Total	31	100

Divorced			Number of psychological	Frequency	Percent
	1	3.2	counseling sessions before amputation		
Widow	8	25.8	0	25	80.6
Total	31	100	1	5	16.1
Residence	Frequency	Percent	3	1	3.2
Urban –	riequency	reiteilt	Total	31	100
municipality/large			. 3	5-	
city	18	58.1			
Urban –small city/			Number of psychological		
town	-	16.1	counseling sessions after amputation	Frequency	Porcont
Rural	5		•	•	Percent
Total	8	25.8	0	11	35.5
Education level	31	100	1	11 _	35.5
Primary education	Frequency	Percent	2	5	16.1
Lower-secondary	4	12.9	3	4	12.9
education					
(gymnasium)	5	16.1	Total	31	100
Vocational school	10	32.3	Accommodation with the new	Frequency	Percent
			body image		
High-school/post-					
secondary education	9	29	Very easy	3	9.7
Higher education	3	9.7	Easy	7	22.6
Total	31	100	Quite difficult	4	12.9
Financial condition	Frequency	Percent	Difficult	6	19.4
Far below average	1	3.2	Very difficult	11	35.5
Below average	10	32.3	Total	31	100
Average	17	54.8	Diagnosis	Frequency	Percent
Above average	3	9.7	Thigh amputation	3	9.7
Total	31	100	Gangrene	4	12.9
Profession/field of			Lower limb amputation		
activity	Frequency	Percent		9	29
Industry	7	22.6	Obliterating arteriopathy of the lower limb	,	12.0
Economy	7	22.0	Atherosclerotic coronary heart	4	12.9
	1	3.2	disease	3	9.7
Trade	2	6.5	Necrotizing fasciitis	1	3.2
Free lancer	1	3.2	Thrombosis	1	3.2
Retired	19	61.3	Ischemia	1	3.2
l la a acada de card	•	3.2	Multiple injury	2	6.5
Unemployed	1	J. 2			-
Total		100	Lobectomy	1	3.2
• •	31	_	Lobectomy Mastectomy	1	3.2 3.2
Total		100	,		3.2
Total Smoker	31 Frequency 16	Percent 51.6	Mastectomy	1 1	
Total Smoker Yes	31 Frequency	100 Percent	Mastectomy Mandible section amputation	1	3.2 3.2

The tests applied to assess the level of depression or anxiety of patients showed a relatively uniform distribution thereof (see **Table 2**). The alpha coefficient for PHQ-9 was 0.892 and for GAD-7 was 0.929, suggesting that the items had a relatively high internal consistency. According to the PHQ-9 test,

29% of the patients presented a "moderate to severe" or "severe" level of depression; Beck's Depression Inventory-Second Edition (BDI-II) test instead showed a share of 16.1% of the patients with severe depression. Severe anxiety was confirmed by the GAD-7 test in the case of 38.7% of the patients.

Table 2. Tests applied to assess the level of depression or anxiety

PHQ-9 (Patient Health Questionnaire-9)	Frequency	Percent
Minimal or none Monitor depression; may not require treatment	7	22.6
Mild depression	7	22.6
Moderate depression	8	25.8
Moderate severe depression	4	12.9
Severe depression	5	16.1
Total	31	100
GAD-7 (General Anxiety Disorder-7)	Frequency	Percent
No anxiety disorder	7	22.6
Mild anxiety	8	25.8
Moderate anxiety	4	12.9
Severe anxiety	12	38.7
Total	31	100
Beck's Depression Inventory-Second Edition (BDI-II)	Frequency	Percent
Minimal depression	11	35.5
Mild depression	6	19.4
Moderate depression	9	29

Patients were also evaluated in terms of quality of their life using the EORTC QLQ-C30 (3rd version) self-report inventory. This patients' assessment tool contained three analytical dimensions: A) global health status, B) functional scales and C) symptom scales/items. In interpreting the results of this instrument, one should take into account that, as mentioned in the manual of the EORTC QLQ-C30 (3rd version), "a high score for a functional scale represents a high/ healthy level of functioning, and a high score for the global health status/ QOL represents a high QOL, but a high score for a symptom scale/

item represents a high level of symptomatology/ problems" [**18**]. The alpha coefficient for EORTC QLQ-C30 (3rd version) was 0.876, suggesting that the items had a relatively high internal consistency.

5

31

A. Global health status - high score for the global health status/ QOL represents a high QOL

The data showed that 63.1% of the patients (i.e., 19 out of 31), self-assessed their health at a level lower than 50 on a scale of o to 100; on the other hand, the highest level of health was 66.7 on the same scale from 0 to 100 (see **Table 3**).

16.1

100

Severe depression

Total

Table 3. GHSscore

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		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	1	3.2	3.2	3.2
	8.33	2	6.5	6.5	9.7
	16.67	6	19.4	19.4	29.0
	25.00	3	9.7	9.7	38.7
	33.33	5	16.1	16.1	54.8
41.67 50.00	41.67	2	6.5	6.5	61.3
	50.00	7	22.6	22.6	83.9
	58.33	3	9.7	9.7	93.5
	66.67	2	6.5	6.5	100.0
	Total	31	100.0	100.0	

B. Functional scales - a high score for a functional scale represents a high/healthy level of functioning

In terms of physical functioning, 71% of the patients were below the level 50 on a scale of 0 to 100, where o showed a very low physical functionality (see **Table 4**).

Table 4. Physical functioning - PFscore

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		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	8	25.8	25.8	25.8
	6.67	1	3.2	3.2	29.0
	13.33	3	9.7	9.7	38.7
	20.00	1	3.2	3.2	41.9
	26.67	2	6.5	6.5	48.4
	33.33	1	3.2	3.2	51.6
	40.00	3	9.7	9.7	61.3
	46.67	3	9.7	9.7	71.0
	53.33	1	3.2	3.2	74.2
	60.00	2	6.5	6.5	80.6
	66.67	2	6.5	6.5	87.1
	73.33	1	3.2	3.2	90.3
8	80.00	2	6.5	6.5	96.8
	86.67	1	3.2	3.2	100.0
	Total	31	100.0	100.0	

The following table shows the extent to which health condition had limited professional activities. 77.4% of the patients

were below the threshold 50 on a scale from o to 100, which means that their health severely limited their role functioning (see **Table 5**).

Table 5. Role functioning - RFscore

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	,00	17	54,8	54,8	54,8
	16,67	2	6,5	6,5	61,3
	33,33	5	16,1	16,1	77,4
	50,00	2	6,5	6,5	83,9
	66,67	4	12,9	12,9	96,8
	100,00	1	3,2	3,2	100,0
	Total	31	100,0	100,0	

Emotional functioning appeared to have been less affected compared to physical or role functioning, with 54.8% of the patients below the level 50 on a scale of 0 to 100 (see **Table 6**).

Table 6. Emotional functioning - EFscore

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	1	3.2	3.2	3.2
	8.33	4	12.9	12.9	16.1
	25.00	3	9.7	9.7	25.8
	33.33	6	19.4	19.4	45.2
	41.67	3	9.7	9.7	54.8
	50.00	4	12.9	12.9	67.7
	66.67	4	12.9	12.9	80.6
	75.00	1	3.2	3.2	83.9
	83.33	1	3.2	3.2	87.1
	91.67	1	3.2	3.2	90.3
	100.00	3	9.7	9.7	100.0
	Total	31	100.0	100.0	

Cognitive functioning was affected to an even lesser extent, 41% of the patients being ranked according to the answers provided,

below the level 50 on the scale from 0 to 100, where 0 represented the minimum level of cognitive functioning (see **Table 7**).

Table 7. Cognitive functioning - CFscore

,						
		Frequency		Percent	Valid Percent	Cumulative Percent
Valid	33.33		6	19.4	19.4	19.4
	50.00		7	22.6	22.6	41.9
	66.67		4	12.9	12.9	54.8
	83.33		5	16.1	16.1	71.0
	100.00		9	29.0	29.0	100.0
	Total		31	100.0	100.0	

Family or social activities appeared to have been significantly affected, 41.9% of the patients being ranked below level 50 on a scale

of o to 100, while for five patients (i.e., 16.1%) social functioning was at a maximum level 100 on the same scale (see **Table 8**).

Table 8. Social functioning - SFscore

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	5	16.1	16.1	16.1
	16.67	2	6.5	6.5	22.6
	33.33	6	19.4	19.4	41.9
	50.00	1	3.2	3.2	45.2
	66.67	6	19.4	19.4	64.5
	83.33	6	19.4	19.4	83.9
	100.00	5	16.1	16.1	100.0
	Total	31	100.0	100.0	

C. Symptom scales/ items - a high score for a symptom scale/ item represents a high level of symptomatology/ problems

In the case of the symptom scale, a higher score represented a negative situation and

problems associated with the amputation condition. Significant fatigue symptoms level, (i.e., over 50% of the cases) were reported in 58.1% of the patients (see **Table 9**).

Table 9. Fatique - FSscore

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	1	3.2	3.2	3.2
	11.11	2	6.5	6.5	9.7
	22.22	1	3.2	3.2	12.9
	33.33	4	12.9	12.9	25.8
	44.44	5	16.1	16.1	41.9
	55.56	3	9.7	9.7	51.6
	66.67	3	9.7	9.7	61.3
	77.78	9	29.0	29.0	90.3
	88.89	1	3.2	3.2	93.5
	100.00	2	6.5	6.5	100.0
	Total	31	100.0	100.0	

Nausea and vomiting had a significant ratio, over 50 on the scale from 0 to 100, where 100 meant a serious condition in the case of only two patients (i.e., 6.4%), which was

normal given that the amputation did not have a direct medical impact on these conditions (see **Table 10**).

Table 10. Nausea and vomiting - NVSscore

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	22	71.0	71.0	71.0
	16.67	5	16.1	16.1	87.1
	33.33	2	6.5	6.5	93.5
	50.00	1	3.2	3.2	96.8
	66.67	1	3.2	3.2	100.0
	Total	31	100.0	100.0	

By contrast, pain was present for 22 of the 31 patients (77.4%). However, we were unable

to exactly control the period and duration of the pain (see **Table 11**).

Table 11. Pain - PSscore

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	1	3.2	3.2	3.2
	16.67	2	6.5	6.5	9.7
	33.33	4	12.9	12.9	22.6
	50.00	8	25.8	25.8	48.4
	66.67	5	16.1	16.1	64.5
	83.33	6	19.4	19.4	83.9
	100.00	5	16.1	16.1	100.0
	Total	31	100.0	100.0	

Only 25.9% of the patients reported dyspnoea above the threshold of 50 on a scale

of o to 100, the remaining ones manifested a low level of dyspnoea (see **Table 12**).

Table 12. Dyspnoea - DSscore

_		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	14	45.2	45.2	45.2
	33.33	9	29.0	29.0	74.2
	66.67	6	19.4	19.4	93.5
	100.00	2	6.5	6.5	100.0
	Total	31	100.0	100.0	

Insomnia was a significant problem (above a level of 50 on a scale from 0 to 100) in 67.8%

of the patients (i.e., 21 of the 31 patients who were part of our survey sample) (see **Table 13**).

Table 13. Insomnia - ISscore

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	6	19.4	19.4	19.4
	33.33	4	12.9	12.9	32.3
	66.67	11	35.5	35.5	67.7
	100.00	10	32.3	32.3	100.0
	Total	31	100.0	100.0	

Loss of the appetite was a significant symptom (on a level above 50 on a scale from 0 to 100) for 41.9% of the patients (see **Table 14**).

Table 14. Appetite loss - ALSscore

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	5	16.1	16.1	16.1
	33.33	13	41.9	41.9	58.1
	66.67	9	29.0	29.0	87.1
	100.00	4	12.9	12.9	100.0
	Total	31	100.0	100.0	

Only 13% of the patients reported a significant level of constipation-related symptoms and only 3.2% experienced significant diarrhea symptoms (see **Table 15, 16**).

Table 15. Constipation - CSscore

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		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	13	41.9	41.9	41.9
	33.33	14	45.2	45.2	87.1
	66.67	2	6.5	6.5	93.5
	100.00	2	6.5	6.5	100.0
	Total	31	100.0	100.0	

Table 16. Diarrhea - DiarScore

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	,00	28	90,3	90,3	90,3
	33,33	2	6,5	6,5	96,8
	66,67	1	3,2	3,2	100,0
	Total	31	100,0	100,0	

Financial difficulties are one aspect that seemed to have marked the majority of the patients who underwent amputation: 58.1% of the patients declared that they faced

financial difficulties at a level above 50 on a scale from 0 to 100 - where 100 was a maximum level of financial difficulties (see **Table 17**).

Table 17. Financial difficulties - FinancialDScore

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		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	5	16.1	16.1	16.1
	33-33	8	25.8	25.8	41.9
	66.67	9	29.0	29.0	71.0
	100.00	9	29.0	29.0	100.0
	Total	31	100.0	100.0	

The table below summarizes the share of patients who manifested a significant level in terms of problems, set at 50 on a scale from o

to 100, for each of the issues considered by the quality of life assessment scale.

Table 18. Patients who manifested a significant level in terms of problems for each of the issues considered by the quality

of life assessment scale

of life assessment scale			
EORTC QLQ-C30	A. Global health status,	B. Functional scales,	C. Symptom scales/ items,
(3 rd version) self-	share of patients with a	share of patients with a	share of patients with a score
report inventory	score <50 on the scale from	score <50 on the scale	>50 on the scale from 0 to 100
	o to 100	from 0 to 100	
Global health status	61.30%		
Physical functioning		71%	
Role functioning		77.40%	
Emotional functioning		54.80%	
Cognitive functioning		41%	
Social functioning		41.90%	
Fatigue			58.10%
Nausea and vomiting			6.40%
Pain			77.40%
Dyspnoea			25.90%
Insomnia			67.80%
Appetite loss			41.90%
Constipation			13%
Diarrhea			3.20%
Financial difficulties			58.10%

The association analysis of the applied test results and the socio-demographic characteristics showed that there were no significant correlations between them. Basically, there is no specific profile or certain characteristics of patients that indicate a high probability of association with a depressive disorder or anxiety. An explanation for this finding may reside in the small number of cases in the sample.

Discussion

There are several limitations of the study. First, there was a small cohort of participants

in the study, which restricted the propensity for complex statistics. the more Αll participants were inpatients of the Department Orthopaedics of and Traumatology, University Emergency Hospital, Bucharest, thus, limited the comprehension of such complex a phenomenon like the quality of life. Limited statistical analysis showed that few factors correlated.

The analysis of the data collected from 31 patients who underwent amputation made it possible to draw a socio-demographic profile in an exploratory manner. Thus, it seems that

amputation mainly occurs in adults/elderly aged over 50 years (80.6% of the patients in our sample galled within this age category). Furthermore. sufferina patients amputations were rather males (64.5% of the total sample), a characteristic confirmed by other studies [2], married, living in urban areas, had a primary, lower-secondary or high school education level and most of them were retied. In terms of certain behaviors associated with an unhealthy lifestyle that could have led to such health problems, the data indicated that alcoholism was not prevalent among such patients - only 16.1% indicated that their relatives suggested they drank too much. Instead, smoking seemed to be a predisposing factor that led to the amputation of a part of the body, given that one in two patients was a smoker (51.6%) and 19.4% were former smokers.

Only one in three patients (32.3%) declared that they adjusted "easily" or "very easily" to their body image, given that 71% of the benefited from psychological counseling, which indicated a high degree of coverage of related support services for this type of patients. This is obviously in relation to another study using a different instrument of evaluation [11], which seems to identify psychological support and time amputation as factors that might influence their adjustment to body image.

The data collected also showed that there were significant shares of patients suffering from mild to severe depression (29% on the PHQ-9 scale, 16.1% according to Beck's Depression Inventory) or severe anxiety (38.7% on the GAD-7) as demonstrated in other studies [3-7,9]. This suggested the need for a more consistent psychological support for patients who have underwent amputation.

EORTC QLQ-C₃o (3rd version) self-report inventory also showed that at least half of the patients had a self-perceived health condition that was deteriorated, their physical and role functioning being primarily affected. Patients who underwent amputation also reported a

significantly high level of "fatigue", "pain", "insomnia" or "financial difficulties".

The study is a first exploratory research on this topic and it allowed the setting-up of a socio-demographic, clinical (from perspective of depressive, anxious) and social (quality of life) profile. However, more laborious statistical analyses to highlight factors favoring the association of amputation with depression or anxiety in certain patients have to be carried out in the future as the sample of patients included in such a research has to be expanded. The estimated difference between the groups of patients that we took into consideration in our sample is highly dependent on the size of sample used. An acceptable probability that there is a statistically significant evidence of a difference between the groups requires a larger sample size than we had at our disposal. It is worth mentioning that questionnaires that evaluate depressive (except for Beck's Depression Inventory) or anxious symptoms are intended for application only in the clinical setting.

Conclusions

The need to provide additional support was also confirmed by the significant share of patients with amputation suffering from depression, anxiety, pain, insomnia or fatigue. In the future, it is important to complete the sample with data collected from new patients and to survey the issues in which these would need patients more effective management (e.g., social relationships, integration into and acceptance by the family and society, reinsertion in the labor market, getting familiar with their legal rights, etc.).

Conflict of Interest statements

Authors state no conflict of interest.

Informed Consent and Human and Animal Rights statements

Informed consent has been obtained from all individuals included in this study.

Authorization for the use of human subjects

Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies, is in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent commitee.

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