Asian Pacific Journal of Environment and Cancer
Vol 7 No 1 (2024), 39-46
Original Research

The Therapeutic Implications of Biomarkers Testing in Non-small Cell Lung Cancer in Middle- and Low-income Country: The Example of Morocco

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Introduction: The Identification of PD-L1, EGFR and ALK status is essential to guide personalized treatment of NSCLC. The objective of our study is to evaluate the implication of changing the therapeutic protocol on the prognosis of Moroccan patients with NSCLC.

Methods: Between January 2019 and February 2023, 96 patients with NSCLC were recruited.

Results: In our population, the patients were treated with different first-line protocols: 83.34% (N=80) with neo-adjuvant chemotherapy, 14.58% (N=14) with immunotherapy and 2.08% (N=2) with targeted therapy. Of the 82 patients who received neo-adjuvant chemotherapy (N=80), 24 were able to switch to immunotherapy. While, the 2 patients who received targeted therapy beforehand also switched to immunotherapy. The influence of the number of chemotherapy (Chemo) cycles on vital status and overall survival (OS) of the 24 patients who switched from their initial protocol to immunotherapy (IO) showed that patients

who received IO after completing the 4 or 5 cycles of chemo had a good OS, with a mean of 29.07 months. In comparison, patients who received less than 4 cycles of chemo had a mean OS of 14.70 months, while those who received more than 5 cycles of chemo had a mean OS of 21.38 months. Furthermore, the association between change in treatment protocol and patient vital status was significant (p=0.013). However, there was a significant difference in OS between patients who maintained the treatment protocol (mean OS=12.43 months) and those who changed the protocol (mean OS=24.01 months) (p=0.000). Multivariate analysis indicated that maintaining the initial therapeutic protocol was independently associated with a reduced OS (p=0.003).

Conclusion: Our results highlight the impact of changing the protocol on the OS of NSCLC patients taking into account the importance of choosing the right timing to switch to IO based on the number of prior chemo cures.

Introduction

In Morocco, lung cancer is the second most common cancer after breast cancer with a prevalence of 13.9% for both sexes and up to 25.6% in men. This cancer is therefore a major a major public health problem [1]. Platinum-based chemotherapy is the first-line treatment for patients with non-small cell lung cancer (NSCLC); however, this treatment has been associated with poor response rates and prognosis [2].

In the last decades, two therapeutic paradigms have become the standard of care in the management of NSCLC, namely targeted therapies and immunotherapy [3]. Targeted therapies are a class of treatments based on the identification of driving genetic changes such as those affecting the epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) genes [3].

These targeted agents like erlotinib, gefitinib, afatinib and osimertinib for patients with EGFR activating mutations [4, 9] and crizotinib, alectinib and lorlatinib for those with ALK rearrangements have been approved for the treatment of metastatic NSCLC which has improved survival in patients expressing ALK-positive tumors with an activating EGFR mutation [5, 6 - 10].

Despite the impressive action of targeted therapies, however, resistance to these treatments develops irreversibly [3]. Therefore, new post-resistance therapeutic strategies will be needed to improve the prognosis of patients with genetic alterations [7]. In addition, a significant proportion of NSCLC patients without genetic alterations can currently be treated with FDA-approved therapies including anti-PD-1 (pembrolizumab, Ketruda) or anti- PD-L1 (atezolizumab,Tecentriq) antibodies. Thus, identification of PD-L1 expression as well as mutations in driver genes including EGFR mutations and ALK rearrangement is essential to predict NSCLC patients who may benefit from personalized therapy [3, 8].

Access to targeted therapies represents significant challenges for patients and clinicians [11] due the excessive cost of the treatment and all eligible patients do not have systematically access to these treatments, especially in our countries. Even when available, paperwork and administrative processes required in order to benefit from targeted therapies and/or immunotherapy are complex and lengthy often resulting in a delayed treatment. In Morocco, access to targeted therapies is partial and due to the delay of administrative procedure by the insurance companies, chemotherapy is still the most used in most patients. Once they are granted access, patients are eventually switched to targeted therapy or immunotherapy. The objective of our study is to evaluate the implication of changing the therapeutic protocol on the prognosis of Moroccan NSCLC patients.

Materials and Methods

Patients

Between January 2019 and February 2023, 96 Moroccan patients with NSCLC were recruited at two different institutions: the Mohamed VI Center for Cancer Treatment of the Ibn Rochd University Hospital in Casablanca and the Ryad oncology Clinic in Casablanca. Eligible participants for our study had to meet the following predefined criteria: age ≥18 years, histologically confirmed NSCLC, having received chemotherapy, immunotherapy, or targeted therapy, with clinical and pathological data, and having a usable tumor sample for determining the PD-L1, EGFR, and ALK statuses. Exclusion criteria applied to patients with other types of lung cancer, unusable tumor samples, and those who had not undergone any therapeutic modalities. The characteristics of patients are cited in Table 1.

Variables	Number (%)
Gender	
Men	83 (86.46)
Women	13 (13.54)
Sex ratio	6.38
Age at diagnosis (years)	
Median [Rank]	67 [38-92]
< 67	44 (45.84)
≥ 67	52 (54.16)
Histological aspect	
Adenocarcinoma	82 (85.42)
Squamous cell carcinoma	14 (14.58) Stage of disease
IIIc	10 (10.42)
IV	86 (89.58)
Performance status (PS)	
PS 0	46 (47.92)
PS 1 -2	50 (52.08)
Smoking status	
Current/ Former	79 (82.29)
Never	17 (17.70)
Metastatic burden	
<3	77 (80.21)
≥3	19 (19.79)
Lymph node metastasis	
No	84 (87.50)
Yes	12 (12.50)
Liver metastasis	
No	87 (90.62)
Yes	09 (09.37)
Bone metastasis	
No	60 (62.50)
Yes	36 (37.50)
Brain metastasis	
No	80 (83.33)
Yes	16 (16.66)
Pleural metastasis	
No	75 (78.12)
Yes	21 (21.87)
Contralateral lung metastasis	
No	61 (63.54)

Yes	35 (36.46)
Adrenal metastasis	
No	71 (73.95)
Yes	25 (26.04)
Expression PD-L1	
TPS<1%	48 (50.00)
TPS 1-49%	23 (23.96)
TPS≥50%	25 (26.04)
ALK Status	
Negative	95 (98.96)
Positive	01 (01.04)
EGFR mutation status	
Wild type	94 (97.91)
Mutant	02 (02.83)
First–line treatment	
Doublet chemotherapy*	80 (83.34)
Immunotherapy**	14 (14.58)
Targeted therapy***	02 (02.08)
Treatment changes	
No	70 (72.92)
Yes	26 (27.08)

Table 1. Characteristics of Patients.

*, Carboplatin and paclitaxel (N=26); Carboplatin and pemetrexed (N=17); Carboplatin and vinorelbine (N=17); Cisplatin and vinorelbine (N=14); Cisplatin and Gemcitabine (N=3); Cisplatin and pemetrexed (N=3).**, Pembrolizumab alone (N=5); Atezolizumab, Carboplatin and paclitaxel (N=4); Pembrolizumab and pemetrexed (N=3); Pembrolizumab, pemetrexed and Carboplatin (N=1); Atezolizumab alone (N=1). ***, Erlotinibe (N=2). Abbreviation, ALK: Anaplasic lymphoma kinase; EGFR: Epidermal growth factor receptor; PD-L1: Programmed death-ligand 1; TPS: Tumor proportion score.

Expression of PD-L1

Tumor expression of PD-L1 was assessed from formalin-fixed, paraffin-embedded (FFPE) tumor samples using the 22C3 pharmDX assay on the Dako link 48 platform.

Tumor cells expressing full or partial membrane staining for PD-L1 were considered positive. Thus, tumor expression of PD-L1 was assessed by the tumor proportion score (TPS) which is defined as the percentage of PD-L1 positive tumor cells (TCs +) in relation to the total TCs.

On the basis of PD-L1 expression, tumor cells were classified into three groups: Negative expression (TPS<1%), low expression (TPS 1-49%), and high expression (TPS≥ 50%). All tumors with TPS≥ 1% were considered PD-L1 positive [12].

EGFR testing

Molecular evaluation of EGFR was performed by qPCR using the cobas® mutation assay for identification of exon 18 (G719A, G719C, and G719S), exon 19, exon 20 (S768I, T790M), and exon 21 (L858R and L861Q) mutations from FFPE tissue. The results revealed the presence or absence of specific mutations of the EGFR gene in the tested samples.

ALK status

ALK translocation assay was performed by immunohistochemistry (IHC) using a rabbit monoclonal rabbit anti-ALK antibody (Clone D5F3, Ventana, Roche). A positive result indicates the presence of strong granular cytoplasmic staining in the tumor cells [13].

Statistical Analysis

All statistical analyses were performed by SPSS version 21 statistical software. The Chi-2 test was used to investigate the association between change in treatment protocol and patient vital status. Kaplan-Meier methods were used to study overall survival according to change in treatment protocol. Comparisons were made using the log- rank test. We also performed univariate and multivariate Cox regression analysis to explore the impact of clinical variables on patients' overall survival. A value of $P < 0.05$ was considered to indicate statistically significant differences.

Results

Characteristics of patients

The characteristics of the 96 patients included in this study are shown in Table 1. In our cohort, 13.54% (N=13) of the patients were women and 86.46% (N=83) were men. The median age was 67 years [38 - 92 years] of which 54.16% (N=52) were 67 years or older. Histological characterization revealed 2 histological types classified as follows: 85.42% (N=82) adenocarcinoma and 14.58% (N=14) squamous cell carcinoma. In addition, 89.58% (N=86) of patients had IV disease at diagnosis. Furthermore, performance status (PS) with a score of 0 was noted in 47.92% of patients (N=46). Regarding smoking status, 17.70% (N=17) of the subjects had no history of smoking. Moreover, 19.79% (N=19) of patients had more than 3 metastatic organs involved (metastatic burden ≥3). Regarding metastatic sites, 12.50% (N=12), 09.37% (N=9), 37.50% (N=36), 16.66% (N=16), 21.87 (N=21), 36.46% (N=35) and 26.04% (N=25) of patients had Lymph node, Liver, Bone, Brain, Pleural, Contralateral lung and Adrenal metastasis, respectively.

The molecular profile of the patients showed the following results: 26.04% (N=25) had a high expression of PD-L1, 1.04% (N=1) had tumors positive for ALK rearrangement and 2.83% (N=2) had tumors with EGFR mutation.

In our population the patients were placed in first line under different therapeutic protocols, either due to the delay of the agreement of immunotherapy and targeted therapy by medical coverage or due to lack of financial means, namely: 83.34% (N=80) patients were placed on neo-adjuvante chemotherapy, 14.58% (N=14) and 2.08% (N=2) of patients were placed on immunotherapy and targeted therapy respectively.

Results of the association between change of treatment protocol and overall patient survival

The results of the analysis of tumor PD-L1 expression according to EGFR/ALK status showed that 96.87% (N=93) of patients had wild-type status (EGFR - and ALK-), whereas 3.13% (N=3) of patients were EGFR (+) or ALK (+) (Table 2).

Variables	Total		PD-L1 expression N (%)	
		TPS <1%	TPS 1-49%	TPS ≥50%
ALK Status				

Negative	95 (98.96)	48 (50.52)	23 (24.21)	24 (25.26)
Positive	01 (01.04)	00 (00.00)	00 (00.00)	01 (100.0)
EGFR mutation status				
Wild type	94 (97.92)	47 (50.00)	23 (24.47)	24 (25.53)
Mutant	02 (02.08)	01 (50.00)	00 (00.00)	01 (50.00)
ALK/EGFR status				
ALK – and EGFR –	93 (96.87)	47 (50.53)	23 (24.73)	23 (24.73)
ALK + or EGFR +	03 (03.13)	01 (33.33)	00 (00.00)	02 (66.67)

Table 2. Tumor Expression of PD-L1 According to EGFR/ALK Status.

Abbreviation, ALK, Anaplasic lymphoma kinase; EGFR, Epidermal growth factor receptor; PD-L1, Programmed death-ligand 1; TPS, Tumor proportion score.

On the basis of the results of molecular tests (tumor expression of PD-L1, EGFR mutation and ALK rearrangement) (Table 2), 72.92% (N=70) of patients were treated with the same therapeutic protocol. However, 27.08% (N=26) had to modify their treatment according to their clinical situation (Table 1).

Among the 80 patients who received first-line chemotherapy, only 24 patients (30%) had received immunotherapy, of which 14 patients (58.33%) have survived to date. To better understand the variability of these results, we examined the vital status and overall survival of the 24 patients according to the number of chemotherapy cycles administered before switching to immunotherapy. The results obtained revealed that patients (N=9) who were switched to immunotherapy after receiving the fourth or fifth cycles of chemotherapy had a better overall survival (mOS = 29.07 months) than those who received fewer than four cycles or more than five cycles of chemotherapy (alive = 7; dead = 2) (Figure 1 and Figure 2).

Figure 1.Study Flow Chart. Abbreviation, ALK, Anaplasic lymphoma kinase; mOS, Mean overall survival; EGFR, Epidermal growth factor receptor; PD-L1, Programmed death-ligand 1.

Figure 2. The Influence of the Number of Chemotherapy Cures on the Vital Status (A) and Overall Survival (B) of Patients who Switched to Immunotherapy.

On the other hand, 56 (70%) continued with the same therapeutic protocol, either because they did not have the financial means to change treatment, or because the molecular tests were negative, or because they had developed incurable metastases, notably bone, liver and brain metastases (Table 1; Figure 1). The two patients placed in first line on erlotinib changed their initial protocol to immunotherapy following tumor progression, but this change did not improve their survival (mean overall survival =16.63) (Figure 1).

The 14 patients who received immunotherapy alone or in combination with first-line chemotherapy (Table 1) maintained their treatment protocol, seven of whom were still alive (mean survival= 16.09 months) (Figure 1).

The analysis of the association between the impact of treatment whether maintained or changed and the status of the patients revealed a significant association (p=0.013) (Figure 3).

Figure 3. Impact of Switching Treatment Protocol (chemotherapy to immunotherapy) on Vital Status.

In addition, the mean 3-year overall survival was 12.43 months for patients who followed their initial treatment protocol. In contrast, patients who opted for a change in treatment protocol had a mean overall survival of 24.00 months with a significant difference between change in treatment protocol and overall survival (Log-rank test, p=0.000) (Figure 4).

Figure 4. Impact of Switching Treatment Protocol (chemotherapy to immunotherapy) on the Overall Survival.

Univariate and multivariate overall survival analysis

In our study, univariate survival analysis revealed several factors significantly associated with unfavorable overall survival such as: histological type (squamous cell carcinoma) (HR: 1.716; 95% CI = 3.327– 10.055; p= 0.002), l performance status (PS 1-2) (HR: 1.582; 95% CI = 06.082 – 12.282; p= 0.002), a metastatic burden greater than 3 (HR: 1.541; 95% CI = 05.764 – 11.803; p= 0.021), the presence of bone metastasis (HR: 1.050; 95% CI = 06.765 – 10.882; p= 0.001), the presence of contralateral lung metastasis (HR: 2.476; 95% CI =14.073 – 23.779; p= 0.020) and maintenance of the initial treatment protocol (HR: 2.644; 95% CI = 18.826 – 29.190; p= 0.001). However, in multivariate analysis, some of these factors retained their importance as independent predictors of unfavorable overall survival. More precisely, the histological type (squamous cell carcinoma) (HR: 3.270; 95% CI = 01.611 – 06.637; p=0.001), the presence of bone metastasis (HR: 0.326; 95% CI = 0.164 – 0.650; p =0.001), and retention of the initial treatment protocol (HR: 3.307; 95% CI = 01.514 – 7.225; p= 0.003). These results highlight the importance of these factors in predicting overall patient survival (Table 3).

Variables	mOS (months)	Univariate survival analysis of OS		Multivariate survival analysis of OS	
		HR: 95% CI	p-value	HR: 95% CI	p-value
Gender			0.663		
Men vs Women (C.REF)	17.971 vs 11.728	2.353: 13.360 – 22.582		Not included	
Age (years)			0.66		
< 67 (C.REF) vs ≥ 67	13.442 vs 18.460	3.002: 12.577 – 24.343		Not included	
Histological aspect			0.002		0.001
Adc (C.REF) vs Squamous CC	19.543 vs 06.691	1.716: 03.327 – 10.055		3.270: 01.611 – 06.637	
Stage of disease			0.308		
IIIc (C.REF) vs IV	13.279 vs 16.629	1.932: 12.842 – 20.416		Not included	
Performance status (PS)			0.002		0.126
PS 0 (C.REF) vs PS 1-2	26.801 vs 09.182	1.582: 06.082 – 12.282		1.671: 0.865 – 3.225	
Smoking status					
Current/Former vs Never (C.REF)	17.704 vs 12.999	2.387: 13.024 – 22.383	0.576	Not included	
Organs involved			0.021		0.561
<3 (C.REF) vs ≥3	20.882 vs 08.784	1.541: 05.764 – 11.803		0.898: 0.625 – 1.290	
Lymph node metastasis			0.693		
No (C.REF) vs Yes	17.954 vs 13.084	2.395: 08.390 – 17.779		Not included	

Liver metastasis			0.411		
No (C.REF) vs Yes	18.933 vs 09.709	2.604: 04.605 – 14.813		Not included	
Bone metastasis			0.001		0.001
No (C.REF) vs Yes	25.786 vs 08.824	1.050: 06.765 – 10.882		0.326: 0.164 – 0.650	
Brain metastasis			0.998		
No (C.REF) vs Yes	18.196 vs 10.879	1.590: 07.762 – 13.996		Not included	
Pleural metastasis			0.951		
No (C.REF) vs Yes	17.077 vs 18.685	4.483: 09.898 – 27.471		Not included	
Contralateral lung metastasis			0.02		0.941
No (C.REF) vs Yes	14.793 vs 18.926	2.476: 14.073 – 23.779		0.974: 0.489 – 1.944	
Adrenal metastasis			0.43		
No (C.REF) vs Yes	19.186 vs 13.039	2.494: 08.150 – 17.928		Not included	
PD-L1 expression			0.115		
<1% (C.REF) vs ≥1%	17.662 vs 14.312	2.676: 09.068 – 19.557		Not included	
Treatment changes			0.001		0.003
No (C.REF) vs Yes	12.438 vs 24.008	2.644:18.826 – 29.190		3.307: 01.514 – 7.225	

Table 3. Predictive Factors of Overall Survival (OS) in Univariate and Multivariate Analysis.

Abbreviation, Adc, Adenocarcinoma; ALK, Anaplasic lymphoma kinase; CC, Cell carcinoma; C.REF, Category reference;.EGFR, Epidermal growth factor receptor; HR, Hazard ratio; IC, Confidence interval; mOS, Mean overall survival; PD-L1, Programmed death-ligand 1.

Discussion

The treatment of non-small cell lung cancer (NSCLC) has evolved considerably in recent years with the use of immunotherapy. Immune checkpoint inhibitors such as anti-PD1 or anti-PD-L1 have improved the therapeutic approach to NSCLC [14]. These inhibitors have become the standard of care for second-line treatment of NSCLC and first-line treatment for patients with high PD-L1 expression [15].

In the NCCN guidelines (National comprehensive cancer network), immunotherapy is also recommended in case of negative results for genetic alterations including EGFR mutations and ALK rearrangement. Thus, accurate identification of biomarkers is essential for personalized disease management [8].

In our study, we limited our objectives to relevant biomarkers for which treatment is validated and available at the Morocco including PD-L1 expression as well as mutations in driver genes such as ALK rearrangement and EGFR mutations. Furthermore, we retrospectively evaluated the impact of protocol change on the overall survival of 96 Moroccan NSCLC patients with established PD-L1, EGFR and ALK status.

Positive PD-L1 expression (TPS ≥ 1%) was observed in 50% of patients of which 23.96% had low PD-L1 expression (TPS 1-49%) and 26.04% expressed it strongly (TPS ≥ 50%) (Table 1).

The results of the Xin Yang and al (2022; China) study are similar to ours with a percentage of

positive PD-L1 expression (TPS≥1%) of 51.8% vs 50% in our study of which 30.3% vs 23.96% had low PD-L1 expression and 21.5% vs 26.04% with high PD-L1 expression [16].

As for the study by Bahnassy and al (2022; Egypt), the positive expression found of PD-L1 (TPS ≥ 1%) was 87.14% [2].

These data suggest that almost half of the patients 50% included in our cohort have a clear indication for immunotherapy treatment in first or second line, which is consistent with current international therapeutic recommendations [17]. These results highlight the importance of considering immunotherapy as an early treatment option for these patients, in order to optimize their prognosis and quality of life. Furthermore, our study highlights the importance of using international treatment guidelines to ensure standardized and effective management of patients with this disease.

Targeted therapy of lung adenocarcinoma with EGFR or ALK tyrosine kinase inhibitors (TKIs) is a significant advance in the treatment of this disease [16]. However, the results in terms of ALK rearrangement (1.04%) and EGFR mutations (2.83%) are relatively low (Table 2) compared with those reported by the study conducted by Seung Eun Lee and al (2019; Korea), where the numbers were 3.4% and 54% for ALK rearrangement and EGFR mutations, respectively [8]. This percentage difference may be explained by the fact that our study included a large percentage of smoking patients (82.29%) (Table 1), who tend to have fewer EGFR mutations and ALK rearrangements than non-smokers. However, it is important to note that these mutations are more common in younger patients and those who have never smoked [10, 18].

The study of the influence of the number of chemotherapy cures on the vital status and OS of patients who switched to immunotherapy showed valuable results which can be described as follows. Of the 24 patients studied, we observed that those (N=9) who received immunotherapy after their fourth or fifth cycle of chemotherapy had a higher OS, with a mean survival time (mOS) of 29.07 months (Figure 2). These results underline the importance of carefully evaluating the right time to switch to immunotherapy, taking into account the number of prior chemotherapy cures. It appears that receiving a limited or an excessive number of chemotherapy cures (less than four or more than five) before switching to immunotherapy may have a negative impact on patients' OS. It should be noted that these results needs to be interpreted with caution, as our study included a small population (N=24). Further studies, involving a larger population and covering a wider range of cases would be required to confirm these findings and obtain more generalizable results. Nevertheless, this initial observation offers important pointers for healthcare professionals in making therapeutic decisions. Identifying the right time to switch to immunotherapy may help improve clinical outcomes and optimize survival for NSCLC patients.

Regarding the association between the impact of treatment protocol changes and patients' vital status and overall survival, our results differ from those of Manirazika et al. (2022; Rwanda). While we observed a significant difference between the protocol change and patients' vital status (p=0.013 vs. p=0.063), a significant association was also revealed between the protocol change and overall survival (p=0.000), which aligns with the results of Manirazika et al. (2022; Rwanda) (p=0.00006) (Figure 3 and Figure 4) [19]. This suggests that the alternative treatment had a notable effect on the health of the studied patients. Although these results are encouraging, further research is needed to confirm them and better understand the factors influencing patient health. These findings have important implications for healthcare, helping professionals make more informed decisions when choosing appropriate treatments for their patients.There are several limitations to this study that should be considered. First, it is important to note that the study was conducted on a relatively small sample, which may limit the significance of the results. Therefore, further research is needed on a larger sample size to confirm the observed trends. In addition, it should be noted that the patients included in the study were treated with different chemotherapy and immunotherapy molecules, which may potentially influence the results obtained. It is therefore essential to continue to explore the effects of different treatments on the results of the analyses.

Finally, it is important to take into account the broadening of the panel of genetic alterations studied, in particular by including genes such as KRAS, BRAF, MET, NTRK. These alterations could indeed play an important role in the understanding of the mechanisms underlying the studied pathology.

In conclusion, our results highlight the impact of switching the protocol on the overall survival of NSCLC patients taking into account the importance of choosing the right timing to switch to immunotherapy based on the number of prior chemotherapy cures. These findings may provide valuable information for clinicians in making therapeutic decisions to improve clinical outcomes for NSCLC patients.

Acknowledgements

Funding statement

We would like to express our gratitude for the financial support that made this research possible. This work was supported by the Laboratory of Cellular and Molecular Pathology, Faculty of Medicine and Pharmacy, Hassan II University of Casablanca.

Compliance and Thesis Approval

We also declare that the thesis of Oussama Aazzane is currently undergoing defense at the Faculty of Medicine and Pharmacy, Hassan II University of Casablanca.

Conflict of Interest

We declare that there are no conflicts of interest to disclose.

Ethics Approval and Participant Consent

This study was approved by the local ethics committee of Ibn Rochd University Hospital (CHU) in Casablanca (Approval number: 03/2022). All patients provided informed consent before participating in the study. Thus, the protocol of our study adheres to the principles outlined in the Helsinki Declaration.

Authors contribution

The authors of this article have made significant contributions to the design, data collection, analysis, and manuscript writing. Their individual contributions are as follows:

Aazzane Oussama: Writing - original version,Conceptualization, Methodology, Data collection. Fathi sofia: Formal analysis, Visualization. Charkaoui Meryem: Formal analysis, Data collection. Acharki Abdelkader, Sahraoui Souha and Benchakroun Nadia: Investigation and Revision. Fellah Hassan and Karkouri Mehdi: Investigation, Supervision, Conceptualization, Methodology, Validation, Revision and Editing.

Availability of data

The data used in this research is available upon request from the authors (Oussama Aazzane,

Hassan Fellah and Mehdi Karkouri).

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