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Table of Contents

Original Articles
Correlations between MHLC Scores and Indicators of Immune Response in Egyptian Women with Breast Cancer .................................................. 07
Eman M. EL-Baiomy, Mohamed L. Salem, Azza El-Amir, Noha A. Sabry, Kenneth A. Wallston, Nehal EL-Mashad

Open label, non–randomized, interventionnal study to evaluate response rate after induction therapy with docetaxel and cisplatin in locally advanced squamous cell carcinoma of oral cavity .......................................................... 12
S. H. Manzoor Zaidi, Ahmad Ijaz Masood, Syed Ijaz Hussain Shah, Irfan Hashemy

The Association Between Clinicopathological Features and Molecular Markers in Bahraini Women with Breast Cancer .................. 19
Aysha AlZaman, Eman Ali, Bayan Mohamad, Moinul Islam, Entisar AlZaman, Yahya AlZaman

Amina Belhadj, Lynda Addou–Klouche, Issam Bouakline, Miloud Medjamia, Hamid Jelloul Benammar, Tewfik Sahraoui

Bibliometric and Comparative Analysis of Castration Resistant and Refractory, Hormone Resistant and Refractory Prostate Cancer Publications ......................................................................................................................... 34
Selahattin Çalışkan, Alkan Çubuk, Abdullah Ilktac

ALK gene rearrangement status in non–squamous non–small cell lung carcinoma in the Middle Eastern population ......................... 38
Samah El Naderi, Rosy Abou–Jaoude, Marc Rassy, Hussein Nasreddine, Elie El Rassy, Claude Ghorra

Micronucleus Test for Diagnosing Uncertain Cases (BI–RADS 3) in Breast Cancer Screening: A Review and Preliminary Results .... 45
Roberto Menicagli, Ortensio Marotta, Roberta Serra

Preoperative Leukocytosis as a Prognostic Marker in Endometrioid-Type Endometrial Cancer: A Single–Center Experience from Saudi Arabia ........................................................................................................ 51
Hany Salem, Ahmed Abu–Zaid, Osama Alomar, Mohammed Abuzaid, Mohammad AlSabban, Tusneem Elhassan, Abdullah Salem,
Yahya Alyamani, Ismail A. Al–Badawi

Review Articles
A Quick Review of Redox State in Cancer: Focus to Bladder ................................................................................................................... 59
Hamid Mazdak, Mehdi Gholampour, Zahra Tolou_Ghamari

Case Reports
Abdominoscrotal Lymphangioma Masquerading as a Communicating Hydrocele: A Case Report ................................................................. 63
Ahmed Al Rashed, Zarine Gazali, Vijay Kumar Malladi, Arbinder Kumar Singal

Hurlthle Cell Adenoma with Micro–Papillary Carcinoma and Parathyroid Adenoma in a Transplant Recipient with Graft Failure: A Case Report ............................................................................................................. 66
Shameema Sharfudeen, Tasneem Amir, Waddah Eskaf, Mahmoud Elsayed Ghanem, Aysha Al Jassar, Kusum Kapila

Feature Article
The State of Cancer Care in the United Arab Emirates in 2020: Challenges and Recommendations, A report by the United Arab Emirates Oncology Task Force .................................................................................................................... 71
Humaid Al–Shamsi, et. al.

Conference Highlights/Scientific Contributions
• Highlights of “Management of Breast and Colorectal Cancer: Recent Updates” Kuwait Conference ......................................................... 88
• News Notes .......................................................................................................................................................................................... 92
• Scientific events in the GCC and the Arab World for 2020 ..................................................................................................................... 96
Original Article

ALK gene rearrangement status in non–squamous non–small cell lung carcinoma in the Middle Eastern population

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Abstract

Background: Worldwide, the frequency of ALK rearrangement ranges between 3–6%, however its prevalence in the Middle Eastern population has not been reported. The aim of this study is to determine the frequency of the ALK rearrangement, as well as the clinico–pathologic characteristics of Lebanese patients with ALK–rearranged lung adenocarcinoma.

Methods: 152 patients diagnosed with non–squamous non–small cell lung carcinomas (NSCLC), at Hôtel–Dieu de France University Hospital between February 2014 and July 2016, were included in the study. ALK gene rearrangement expression was screened by immunohistochemistry (IHC) (D5F3 Clone). Positive cases were then sent for confirmation with Fluorescence in situ hybridization (FISH) technique.

Results: On immunohistochemistry, patients were distributed as following: score 0: n=108, score 1+: n=26, score 2+: n=9, score 3+: n=9. ALK gene rearrangement was detected in 6 out of 18 (2+ and 3+ score) tested patients by FISH technique. The presence of ALK rearrangement was significantly associated with the female gender (n=6, p=0.003) and with non–smoking status (n=4, p=0.0.18).

Conclusion: This study confirms that the prevalence of the ALK gene rearrangement in the Middle Eastern region is within the worldwide ranges and is almost exclusive to patients with adenocarcinoma subtype and tends to occur more frequently in women and non–smokers.

Keywords: epidemiology; ALK rearrangement; lung adenocarcinoma; Middle East; Lebanon

Background

Lung cancer is the third most frequent cancer in incidence and the leading cause of cancer–related mortality (¹,²). Non–small cell lung cancer (NSCLC), constitutes the largest subgroup and have been recently classified into squamous and non–squamous NSCLC for clinical purposes. Interestingly, the recent reports have shown a drastic increase in adenocarcinoma prevalence that is probably attributed to the modifications in smoking habits (³,⁴). The recent advances in the treatment of NSCLC have led to a mandatory identification of targetable driven mutations, among them the Anaplastic lymphoma kinase (ALK) fusion gene. It involves most frequently the ALK gene (2p23.2) and the EML4 gene (Echinoderm microtubule–associated protein–like) (2p21)⁵–⁸. This mutation occurs in 3–6% of patients with lung adenocarcinoma, predominantly in younger patients, never or light smokers, in females and at a metastatic stage. Moreover, this molecular alteration is mutually exclusive with EGFR mutation (⁹–¹³). We have previously reported on the occurrence of EGFR mutations in Lebanon and have shown similar results to those in the Caucasian populations. Herein, we report on the prevalence of ALK rearrangement. We further describe the clinical and histopathological characteristics of these ALK–positive patients in comparison to other studies reported in the literature.

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Methods

Clinical and histopathologic data

The study was conducted on a retrospective series of 152 non–squamous NSCLC patients for whom ALK gene rearrangement testing was carried out in the Pathology Department of Hôtel–Dieu de France University Hospital, between February 2014 and July 2016.

All patients with confirmed non–squamous NSCLC were eligible for this analysis. We excluded patients with missing data. The demographic and clinical information of patients including age, gender, smoking status, and disease imaging at diagnosis were retrieved from medical records. All tumors were restaged as per the 8th TNM staging of lung cancer and categorized into localized, locally advanced and metastatic disease. Patients were also categorized according to the histologic subtype, the sample type (biopsy, cytology, or surgical resection), the site of sample (primary lung tumor or metastatic tumor), and immunostaining status of TTF-1 and/or Napsin A.

Technique

Tissue samples were fixed in 10% neutral formalin and embedded in paraffin (FFPE). Paraffin blocks were then cut at a thickness of 3–5 µm. Immunostaining was performed using an anti–TTF-1 antibody (Thyroid Transcription Factor–1) (4:100 dilution; Biogenex; Clone BGX–397A) on the Ventana Benchmark GX Autostainer, and Napsin A (1:400 dilution Novocastra; clone IP64) on the Dako Autostainer Link 48.

Upon confirmation of the non–squamous NSCLC diagnosis, the 152 patients were tested for ALK gene rearrangement by immunohistochemistry (IHC) with the pre–diluted anti–ALK antibody (Ventana®; clone D5F3), on the Benchmark GX autostainer, according to the manufacturer’s recommendations. The ALK–IHC result was determined according to the intensity of staining using a scoring system consisting of: 0 (no staining), 1+ (minimal staining), 2+ (moderate staining), 3+ (strong staining). Positive and negative controls were used. For each case, a slide was stained with a matched Rabbit Monoclonal Negative Control Ig antibody. Positive controls included ALK rearranged FISH positive lung tumor sample, as well as appendix tissue. Negative controls included ALK negative lung tumors.

When ALK–IHC was 2+ (equivocal result) or 3+, FISH was carried out at BERGONIE Institute – Bordeaux, FRANCE for confirmation. FISH was considered positive if 15% or more of the cancer cells showed split red and green signals separated with 2 or more signal diameters, or single red 3’ signals (combined or not to fused and/or broken–apart signals). Other FISH findings were considered positive if 15% or more of the cancer cells showed split red and green signals separated with 2 or more signal diameters, or single red 3’ signals (combined or not to fused and/or broken–apart signals). Other signal patterns were considered FISH negative. Definitive ALK gene rearrangement status was defined according to the gold standard FISH results. At each point, two pathologists independently scored each case as per our department policy and disagreements were resolved by discussion.

Statistical analysis

SPSS v23 software was used for statistical analysis. Our clinical data are expressed in mean ± standard deviation (SD), or median, or percentage. The relationships between each of the variables are assessed by Pearson’s correlation. A two–tailed p value <0.05 is considered significant.

Results

Clinicopathologic characteristics of patients

The mean age of the 152 patients was 65.5 ± 10.5 years (range 29–87 years). The majority were males 61.8% (n=94) and former or current smokers 78.9% (n=120). At diagnosis, the staging of the patients identified a majority of 71.7% (n=109) with metastatic disease. The ALK gene rearrangement testing was carried out mainly on the primary pulmonary tumor (82.9%, n=126). The rest of the cases (17.1%, n=26) corresponded to different metastatic localizations of the pulmonary tumor.

Concerning histological features, adenocarcinoma was the major histopathological subtype found in 91.4% (n=139). Positive immunostaining of TTF-1 and/or Napsin A was observed in 71% (n=108). ALK gene rearrangement was detected in 6 out of the 152 patients (3.9%) by the FISH technique (Table 1).

Clinicopathologic characteristics according to ALK status

The ALK–IHC testing showed score 2 in 9 cases and score 3 in 9 cases; among them, FISH positivity was detected in 2 cases and in 4 cases respectively (Figure 1). Cases with 0 and 1+ scores were considered negative. The ALK gene rearrangement was finally detected in 6 patients. All of them were females (p=0.003), with an average age of 58.5 years. They had metastatic disease at diagnosis and 4 were non–smokers (p=0.018) (Table 2).
ALK gene rearrangement in the Middle Eastern population, Samah El Naderi, et. al.

Testing overcomes this limitation with several adjunctive advantages, being a cost-effective and rapid technique. In line with these benefits, the VENTANA ALK (D5F3) CDx Assay provides a standard scoring scheme and is FDA approved (15–18). However, the optimal screening approach

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Mean ± DS</th>
<th>65.5 ± 10.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td></td>
<td>[29 – 87]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gender, % (n)</th>
<th>Female</th>
<th>38.2% (58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>61.8% (94)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Smoking status, % (n)</th>
<th>Never smoker</th>
<th>21.1% (32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current or former smoker</td>
<td>78.9% (120)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disease stage, % (n)</th>
<th>Localized</th>
<th>11.8% (18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locally advanced</td>
<td>16.5% (25)</td>
<td></td>
</tr>
<tr>
<td>Metastatic</td>
<td>71.7% (109)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample origin, % (n)</th>
<th>Pulmonary</th>
<th>82.9% (126)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra pulmonary</td>
<td>17.1% (26)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample type, % (n)</th>
<th>Surgical</th>
<th>22.4% (34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td>74.3% (113)</td>
<td></td>
</tr>
<tr>
<td>Cytology</td>
<td>3.3% (5)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample source, % (n)</th>
<th>Hôtel–Dieu de France</th>
<th>65.8% (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Referred</td>
<td>34.2% (52)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histopathological subtype, % (n)</th>
<th>Adenocarcinoma</th>
<th>91.4% (139)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC</td>
<td>3.3% (5)</td>
<td></td>
</tr>
<tr>
<td>Adenosquamous Carcinoma</td>
<td>2% (3)</td>
<td></td>
</tr>
<tr>
<td>MANEC</td>
<td>1.3% (2)</td>
<td></td>
</tr>
<tr>
<td>Undifferentiated large cell carcinoma</td>
<td>1.3% (2)</td>
<td></td>
</tr>
<tr>
<td>Large cell neuroendocrine carcinoma</td>
<td>0.7% (1)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IHC: TTF–1/Napsin A, % (n)</th>
<th>TTF–1+ and/or Napsin A+</th>
<th>71% (108)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTF–1– and Napsin A–</td>
<td>21.1% (32)</td>
<td></td>
</tr>
<tr>
<td>IHC unavailable</td>
<td>7.9% (12)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ALK gene rearrangement, % (n)</th>
<th>ALK positive</th>
<th>3.9% (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK negative</td>
<td>96.1% (146)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1: Patients repartition according to clinical and pathological characteristics**

**Discussion**

Until before January 2018, the FISH technique was the gold standard for detecting ALK gene rearrangement. However, this technique may not detect variants of the ALK rearrangement fusion gene (14,15). The ALK IHC testing overcomes this limitation with several adjunctive advantages, being a cost–effective and rapid technique. In line with these benefits, the VENTANA ALK (D5F3) CDx Assay provides a standard scoring scheme and is FDA approved (15–18). However, the optimal screening approach
remains to be determined as no optimal screening—diagnosis paradigm has been assigned (15,19,20). Indeed, some studies have shown that patients with a FISH negative/IHC positive ALK profile had good response to ALK— inhibitors (20,21). Recently, the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC) and the Association of Molecular Pathology (AMP) published updates concerning molecular testing guidelines and recommended using IHC as an equivalent alternative to FISH for ALK testing (22).

Staining artifacts may interfere with the interpretation of the results whether by IHC or FISH. To overcome this problem, such cases should subsequently be tested by another validated method like RT–PCR and NGS (22).

The adopted screening algorithm seems to differ between countries which may affect the detection of the ALK gene rearrangement and subsequently its incidence (Table 3). The European experience showed variable results with 2.2–5% ALK— rearrangement as defined by FISH. In one study using FISH to confirm ALK— IHC positivity, narrowed its occurrence from 6.2 to 2.2% (23,24). In the United States (USA) experience, where all patients got tested with the two techniques alternately, a prevalence of 5.6% was obtained with neither FISH nor IHC by itself able to identify all cases (25). The published Eastern experience used the IHC technique and has shown 3–9.5% prevalence (26–28). The data from North Africa and Middle East is sparse with only two small studies using FISH, the first from Egypt reporting 7.3% and the second from the Levant area reporting 1.9% of ALK— gene rearrangement (29,30).

Unlike the eastern data, the western publications correlated the presence of ALK— gene rearrangement to younger age, non— smoking status, and pure adenocarcinoma (23–28). The nearest geographic location to Lebanon, Egypt and countries of the Levant area, had results in line with the eastern data (29,30). To our knowledge, our results are the first from the Middle Eastern region that confirmed the western results.

In our study, it is imperative to state that of the 9 patients with ALK—IHC 3+, 3 were FISH— negative. An RT—PCR study was performed for 2 of these 3 patients, confirming ALK positivity in 1 case. Furthermore, 1 of the 2 RT—PCR negative patients was given an ALK inhibitor and showed a partial response (conforming to RECIST v1.1) with radiological stabilization of the disease. Accordingly, these 2 “false negative” patients (IHC 3+ and FISH negative) were sorted as ALK— positive by dint of IHC and were potentially eligible for ALK— inhibitors. The presence in our cohort of those 3 patients with “discrepant” results (ALK 3+ in IHC, and FISH—) points out the necessity of ALK—IHC screening. This is especially important, given that response to ALK— inhibitors has been documented in the literature in both IHC+/FISH— and IHC—/FISH+ patients (20,31).
Conclusion

The patients harboring the ALK fusion gene were predominantly females, non-smokers, and had metastatic disease. The ALK gene rearrangement tended to be almost exclusively expressed in patients with lung adenocarcinoma. Our results confirmed the similarities of the epidemiologic and pathologic data between Lebanon and the Western countries for ALK gene rearrangement. To date, no consensual algorithm is recommended for the detection of ALK gene rearrangement. Such guidelines would standardize the practices and guarantee a better selection of the patients likely to benefit from ALK-inhibitors.

Acknowledgement

Author’s contribution

Samah El Naderi: outline, design of the article, corrections and final approval; Rosy Abou–Jaoude: review of literature, collection of data, drafting and critical writing; Marc Rassy: statistical analysis and results interpretation;
Hussein Nasreddine: collection of data; Elie Rassy: review of literature and critical writing; Claude Ghorra: concept, design, corrections and final approval.

Compliance with Ethical Standards

Research involving Human Participants and/or Animals: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.”

Informed consent: Not needed as none of the participants had identifying information included in this article.

References


Table 3: Prevalence of ALK rearrangement in different geographic regions

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of patients</th>
<th>ALK+ (%, n)</th>
<th>Technique</th>
<th>IHC (%, n)</th>
<th>FISH (%, n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lebanon (our series)</td>
<td>2017</td>
<td>3.9% (6)</td>
<td>IHC (%, n)</td>
<td>11.8% (18)</td>
<td>FISH (%, n)</td>
</tr>
<tr>
<td>Levant Area</td>
<td>2017</td>
<td>1.9% (3)</td>
<td></td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Egypt</td>
<td>2015</td>
<td>7.3% (8)</td>
<td></td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Europe</td>
<td>2014</td>
<td>6.2% (80)</td>
<td></td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>France</td>
<td>2016</td>
<td>4.8% (88)</td>
<td></td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>USA</td>
<td>2009</td>
<td>5.6% (20)</td>
<td></td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>China</td>
<td>2016</td>
<td>4% (93)</td>
<td></td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Japan</td>
<td>2010</td>
<td>3.1% (8)</td>
<td></td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Korea</td>
<td>2012</td>
<td>9.5% (25)</td>
<td></td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

FISH: fluorescence in situ hybridization; IHC: immunohistochemistry; NR: not reported


18. FDA–Approved ALK IHC CDx is Superior to Another IHC Assay for Patient Selection of ALK Inhibitor Treatment | International Association for the Study of Lung Cancer [Internet]. [cited 2018 Feb 26]. Available from: https://www.iaslc.org/articles/fda-approved-alk-ihc-cdx-assay-for-patient-selection-of-alk-inhibitor


