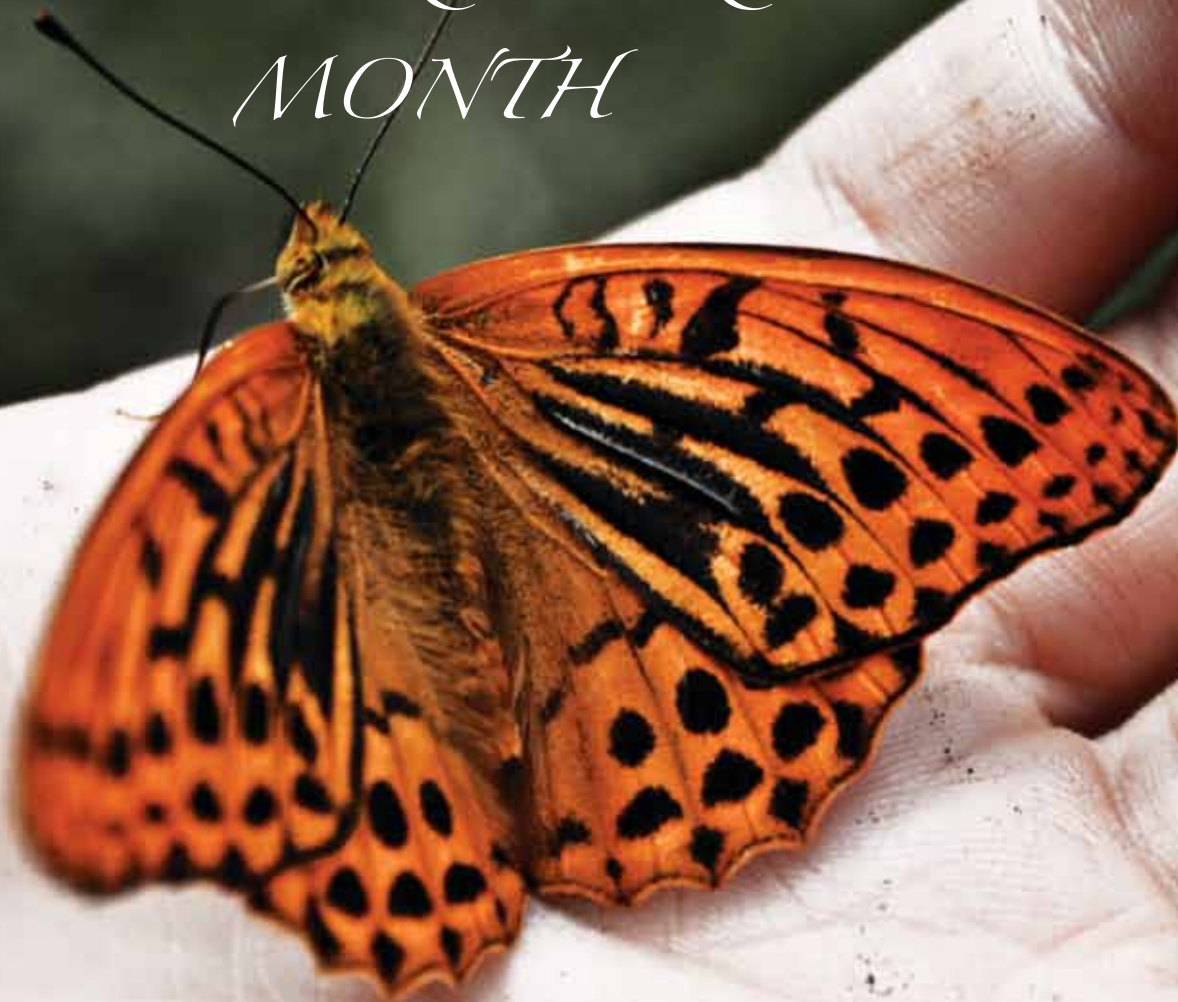


CANCER SURVIVOR MONTH



Original Articles

- L'approche immuno-ptoteomique SEPRA et Cancer du Sein (in French)
- Receptor for hyaluronic acid-mediated motility (RHAMM/CD168) in AML patients
- Egyptian experience of modified medical thoracoscopy

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AMAAC Introduction

The Arab Medical Association Against Cancer (AMAAC) is a medical body that was established in 2001 as part of the Arab Medical Association where its main office is located in Cairo - Egypt, and it is also a continuation of the Arab Council Against Cancer that was founded in 1995. The Executive Committee of (AMAAC) is represented by two members who are named officially by the Oncology Society of each Arab Country.

The Arab Medical Association Against Cancer aims at strengthening relationships between members in different Arab Countries to raise the level of cooperation in the field of oncology on both scientific and practical aspects. Exchanging information and researches between members through Regional and Arab Conferences and Publications. Holding Public Awareness Campaigns in the field of oncology that are organized by Arab Countries. Participating in scientific activities with International Oncology Societies. Finally, encouraging researchers and doctors to meet and exchange experiences together with finding training opportunities in the field of oncology inside and outside the Arab World.

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L'approche immuno-protéomique SERPA : définition et intérêt biomédical dans les cancers du sein

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Abstract

Le cancer du sein représente la première cause de mortalité féminine. Il est défini comme étant une prolifération anormale et anarchique des certaines cellules. L'expression de nombreuses protéines est altérée lors de la transformation cancéreuse et lors de la progression tumorale. Cette expression protéique peut déclencher une réponse d'autoimmunité et la libération d'autoanticorps qui semblent avoir un rôle cruciale dans le pronostic, le diagnostic que pour la création de nouvelles cibles thérapeutiques potentielles. Aujourd'hui l'approche immuno-protéomique SERPA (SERological Proteomic Analysis) permet la recherche simultanée d'autoanticorps et l'identification des épitopes antigénique immunodominant. Cette technique présente l'immense avantage d'être rapide et spécifique. Dans cette revue nous avons mis l'accent sur l'apport de cette approche immuno-protéomique au niveau des cancers du sein d'une part, et d'autre part à la découverte de nouveaux marqueurs de diagnostiques et de pronostiques.

Introduction

Le cancer du sein est un problème de santé publique majeur, à la fois dans les pays développés ou en cours de développement (1). C'est une maladie multifactorielle impliquant à la fois des facteurs génétiques et des facteurs liés à l'environnement. Cette pathologie est la conséquence de dérèglements dans la machinerie cellulaire qui résultent de l'activation de gènes menant à la prolifération cellulaire (oncogènes), de l'inhibition de gènes contrôlant de façon négative cette prolifération (gènes suppresseurs de tumeurs) ou de dérèglements affectant ces deux mécanismes à la fois (2). Le cancer du sein est une prolifération anormale des cellules dans la glande mammaire. La perte du contrôle de cette prolifération aboutit à une croissance cellulaire anormale et à une multiplication cellulaire très importante qui dépasse la mort cellulaire. Ces caractères se transmettent d'une génération cellulaire à une autre d'où l'apparition d'un clone de cellules immortelles. Ce clone de cellules indésirables finit par former un nodule appelé tumeur (3) and (4).

La détermination et la compréhension des phénomènes liés à la carcinogénèse nécessitent une approche génétique qui permettra de caractériser les différents gènes impliqués dans la prolifération tumorale et de déchiffrer les voies de signalisation intracellulaires menant au déclenchement et à la progression des tumeurs (5). Parallèlement, des études protéomiques, s'imposent et permettront d'analyser et

de caractériser des marqueurs protéiques impliquées dans le développement tumoral (5). Ces études protéomiques sont fondées sur des techniques des séparations d'échantillons protéiques complexes, telles que l'électrophorèse bidimensionnelle (2-DE), la technique SERPA (SERological Proteomic Analysis), et la spectrométrie de masse de type MALDI-TOF : Matrix Assisted Laser Desorption Ionisation Time Of Flight (6). La spectrométrie de masse permet depuis une dizaine d'années de répondre à de nombreuses questions biologiques, notamment, concernant l'identification des marqueurs protéiques et leur caractérisation (modifications post-traductionnelles, modifications chimiques, mutations d'acides-aminés...). Ainsi, la spectrométrie de masse couplée à l'électrophorèse bidimensionnelle, au sein de l'approche protéomique ont évolué afin de devenir un outil puissant de choix (7). En effet, la spectrométrie de masse permet d'obtenir des informations sur les masses des peptides intacts, mais aussi des informations de séquences par spectrométrie de masse en tandem (MS/MS), indispensables pour confirmer les identifications litigieuses mais aussi pour caractériser les peptides de protéines n'appartenant pas aux banques de données protéiques (7). Plusieurs études protéomiques permettraient de caractériser de nouveaux marqueurs protéiques qui faciliteraient le diagnostic précoce du cancer du sein et aideraient à déchiffrer les différentes voies de signalisation intracellulaires menant au déclenchement et à la progression des tumeurs. De telles données devraient permettre l'élaboration de nouvelles cibles thérapeutiques et mener au développement de stratégies innovatrices pour combattre le cancer (5).

La recherche de marqueurs protéiques tumoraux et sériques

Afin d'élucider les phénomènes liés à la carcinogénèse, des études de génomique sont indispensables pour caractériser les différents gènes ainsi que les voies de signalisation intracellulaires menant au processus tumoral. Parallèlement, des études de protéomique permettront de comprendre les relations structure-fonction des différentes protéines impliquées dans ce processus tumoral et l'identification de nouveaux marqueurs pathologiques (7). Par définition, un marqueur tumoral est une molécule présente dans l'environnement tumoral et qui fait appel aux notions de spécificité, de sensibilité et de valeur prédictive positive. Sa concentration devrait montrer une corrélation entre le taux du marqueur et la taille de la tumeur. Plusieurs travaux ont abordé la caractérisation de nouveaux marqueurs protéiques cellulaires et sériques associés au développement de cancer du sein, moyennant la 2-DE couplée à la spectrométrie de masse qui pourraient être associés au

développement des cancers mammaires (Tableau 1) (7). Une étude protéomique réalisée par Roberts et al. (8) a montré une surexpression du récepteur d'estrogène (RE) dans le cadre du cancer mammaire. Ce marqueur protéique a démontré le premier impact dans la prise en charge thérapeutique des cancers du sein. Les travaux de Hondermarck H et al. (7) ont montré, dans le cadre de cancer du sein, la surexpression, de certains marqueurs protéiques telles que la protéine Glucose Regulated Protein (GRP 78), la calreticuline, la tropomyosine 3 et la vimentine. Une autre étude protéomique menée par Vercoutter-Edouart et al. (9) a permis de caractériser la protéine chaperonne moléculaire 14-3-3 sigma, comme marqueur potentiel précoce de la cancérisation mammaire. La forme 14-3-3 sigma a été fortement inhibée dans les cancers du sein ainsi que dans des lignées cellulaires tumorales, MCF-7 et MDA-MB-231. Ces résultats ont permis d'attribuer le rôle de suppresseur de tumeurs à cette protéine (9). Une autre étude protéomique ayant utilisé la 2-DE couplée à la spectrométrie de masse a été appliquée sur des lignées mammaires (MCF-10A, BT474, and MDA-MB-468). Cette étude a montré une forte expression de kallikreines 5, 6 et 10 en tant que biomarqueurs du cancer du sein (10). Une étude protéomique combinant la 2-DE/MALDI-TOF, dans le cadre du cancer mammaire, a montré la surexpression de plusieurs protéines du cytosquelette à savoir, la vimentine, la β 5-tubuline et l'actine G (14). Ces protéines semblent être impliquées dans l'invasion tumorale (11). Imai et al. (12) ont montré, dans le cancer du sein, la surexpression de protéines telles que, l'annexine-2, la galectine-1 et la tropomyosine-1. Cette étude a montré aussi, que ces marqueurs semblent avoir un rôle dans la progression tumorale et peuvent être utilisés en tant que des cibles thérapeutiques potentielles (12). Une étude récente visant à identifier de marqueurs potentiels au niveau du cancer mammaire et employant la 2-DE couplée au MALDI-TOF MS, les sérums de 54 contrôles et de 76 patientes atteintes d'un cancer mammaire ont été comparés. Cette étude a mis en évidence une expression différentielle de HSP27 et de 14-3-3 sigma dans le sérum des patientes atteintes d'un cancer mammaire (13). Ricolleau et al. (14) ont appliqué la méthode des puces à protéines à la caractérisation de marqueurs utiles au pronostic du cancer du sein. Cette étude a montré une surexpression de l'ubiquitine et de la chaîne légère de la ferritine (14).

La recherche d'auto-anticorps moyennant l'approche serpa

De nouvelles technologies sont apparues récemment, aboutissant au concept de la détection de nouveaux marqueurs protéiques potentiels. L'immunocriblage est basée généralement sur l'approche SERPA qui permet l'analyse des modifications globales du répertoire d'autoanticorps et la découverte de nouveaux marqueurs tumoraux sériques utiles aussi bien pour le diagnostic, le pronostic que pour le suivi thérapeutique de la maladie (6). L'analyse sérologique d'autoanticorps par cette approche, se définit comme étant une combinaison de 2-DE, le transfert des protéines cellulaires et tumorales séparées sur membrane immobilisante telle que la membrane PVDF (polyvinyl difluorure), support d'une réaction antigène/anticorps avec les sérums de malades et témoins, suivi de l'analyse comparative des gels et de l'immunoblot (Figure 1). La caractérisation des antigènes sera établie généralement par la spectrométrie de masse (6). La technique protéomique SERPA présente l'immense avantage d'être rapide et spécifique. Cette technique permet, non seulement la recherche simultanée de toutes les autoanticorps, en une seule fois, mais aussi l'identification des épitopes antigénique immunodominant (6). Actuellement la recherche d'autoanticorps au niveau du sérum est une voie de recherche très attrayante. Des anticorps dirigés contre des immunodéterminants antigéniques néoplasiques sont mis en évidence dans les cancers du sein. L'approche immuno-protéomique SERPA permet l'analyse du répertoire d'autoanticorps ainsi la découverte de nouveaux marqueurs tumoraux sériques à utilité médicale (6).

Cette approche immuno-protéomique, a permis d'identifier des antigènes tumoraux mammaires qui sont capables de déclencher une réponse immunitaire humorale. Ces marqueurs spécifiques pourraient par la suite, être utiles aux diagnostics et aux pronostics des cancers mammaires (6). Bien que la détection d'autoanticorps soit une approche encore nouvelle en cancérologie, elle fait partie aujourd'hui de la stratégie diagnostique et pronostique de cancer du sein (6). Cette étude a permis de montrer la présence d'auto-anticorps de type IgG dirigés contre des protéines impliquées dans le processus tumoral. L'identification de marqueurs tumoraux impliqués dans le développement de la pathologie mammaire a permis de définir et d'identifier autant de cibles thérapeutiques potentielles pour la mise au point de médicaments, que d'acteurs cruciaux pour le diagnostic et le pronostic de cette maladie. Plusieurs travaux se sont inscrits dans cette voie d'immunocriblage. Les autoanticorps anti-P53 sont décrits comme étant surexprimés dans de nombreux cancers, à savoir, le cancer du sein, le cancer colorectal et le cancer de la langue (15) and (16).

D'autres études ont montré que ces autoanticorps anti-P53 sont dus généralement, à une altération de la protéine P53 au cours de la tumeur et que la concentration sérique des anticorps anti-P53 est corrélée avec la surexpression de la protéine P53 altérée dans la tumeur (17). Une autre étude menée par Le Naour F et al. (6) a permis de caractériser la protéine RS/DJ1, comme marqueur potentiel des cellules tumorales du sein. Il a été démontré que cette protéine est capable de déclencher une réaction d'autoimmunité et à la production d'autoanticorps anti-RS/DJ1. Une autre étude protéomique a été appliquée par la même équipe 6 sur les sérums des patients ayant un cancer du foie, a montré plusieurs antigènes qui sont capables de déclencher une réponse d'autoimmunité, à savoir, la calreticuline, la Hsp 60, la β -tubuline, la cytokératine 8, la cytokératine 18, la F1-ATP synthase et la créatine Kinase β . Ces protéines connues participent à différentes fonctions biologiques comme l'architecture du cytosquelette, le stress cellulaire et le métabolisme intermédiaire (6). Dans le même cadre, une étude récente de Zimmermann et al. (18) a montré l'expression d'autoanticorps anti-cardiolipine et anti-beta2 glycoprotéine-1 dans le cadre du cancer mammaire. Les mécanismes précis qui gouvernent cette auto-immunisation ne sont pas encore élucidés. Récemment, une étude protéomique (SERPA), a été réalisée sur le cancer du sein de type carcinome canalaire infiltrant, a montré pour la première fois une forte expression d'auto-anticorps ciblant des protéines de stress oxydatif, à savoir, la peroxyredoxine 2, la protéine disulfide isomérase et la manganèse superoxyde dismutase (MnSOD), des protéines chaperonnes, la HSP60 et la β cristalline. Une protéine suppresseur de tumeur, la prohibitine, et des protéines impliquées dans l'architecture du cytosquelette telles que la cytokératine 8, la cytokératine 18 et la β tubuline (19). La peroxyredoxine 2, et des membres de la famille des heterogeneous nuclear ribonucleoprotein (hnRNP), à savoir, la hnRNPk et hnRNP A2/B1, protéines impliquées dans l'apoptose, l'épissage et le transport d'ARNm du noyau vers le cytoplasme (19). La peroxyredoxine 2, protéine cytosolique, joue un rôle anti-oxydatif contre le stress oxydant 20. Une protéine comme la peroxyredoxine 2, a un rôle important dans la régulation et le contrôle de la transformation oncogénique de l'épithélium mammaire, car elle est impliquée dans la protection de cellules mammaires contre les oxydations radicalaires et permet aussi de moduler la prolifération cellulaire et l'apoptose de certaines cellules malignes (20). A nos jours, peu d'études qui ont mentionné la présence d'autoanticorps contre les peroxyredoxines chez des sujets ayant une malignité. Ces études rétrospectives portant sur la détection d'autoanticorps dirigés contre la peroxyredoxine, ont montré l'importance de ces autoanticorps comme étant des marqueurs de diagnostic, de pronostic, et qui serait d'un grand apport dans la prévention de la maladie. Une étude protéomique réalisée par Fujita et al. (21) ont montré que la peroxyredoxine VI se comporte comme étant un marqueur protéique potentiel de pronostic chez les patientes souffrant d'un cancer du

l'œsophage (21). L'approche SERPA montre de même la présence d'autoanticorps contre le manganèse superoxyde dismutase (MnSOD) et la protéine disulfide isomérase (PDI) chez les sérums des patientes souffrant d'un cancer du sein (20). La MnSOD, est une enzyme mitochondriale qui fait partie de l'arsenal défensif, qui protège les cellules contre des espèces réactives (ROS). Cette enzyme protège l'ADN et les membranes cellulaires d'un dommage destructif causé par le stress oxydatif (22). Plusieurs travaux portant sur la détection d'autoanticorps dirigés contre certains antigènes tumoraux ont mis en évidence des autoanticorps anti-MnSOD (22) and (23). Ces autoanticorps ont été utilisés comme étant des marqueurs de diagnostic utile pour cette pathologie maligne (22). Bien que les mécanismes biologiques qui sont responsables de la synthèse d'autoanticorps chez les patients cancéreux demeurent inconnus, leur présence a été suggérée au pouvoir pathogène de ces protéines dans les tissus tumoraux (24). D'autres travaux suggèrent que cette augmentation puisse être un facteur de contribution de leurs immunogénités (22). Des autoanticorps anti-protéines chaperonnes ont été de même identifiées dans le cancer du sein par l'approche SERPA, à savoir, la HSP 60 et la cristalline β (20). Les protéines chaperonnes HSP « Heat Shock Protein » sont des protéines ubiquitaires hautement conservées au cours de l'évolution. Ces protéines chaperonnes aident la cellule à vivre et à fonctionner et permettent aussi l'adressage des protéines dans le compartiment cellulaire adéquat. Récemment, une étude protéomique a signaler que la surexpression de la protéine HSP60 au niveau de cancer du sein, peut avoir une valeur pronostique d'autant qu'elle est corrélée avec la présence d'un métastase ganglionnaire (25). Ces données rapportées ainsi confirment que les changements de la conformation protéique mènent dans le cas du cancer du sein à une réaction d'autoimmunité. Ces activités immunologiques sont liées généralement aux systèmes des repliements protéiques qui puissent être un marqueur important dans la carcinogenèse mammaire. Des autoanticorps anti-protéines chaperonnes autres que la HSP60 ont été rapportées dans la malignité du sein (26). Parmi ces protéines chaperonnes qui déclenchent une réaction d'autoimmunité au niveau de cancer du sein, on note la protéine HSP90, dont la surexpression d'autoanticorps est associée avec un faible taux de survie (27). Dans le cancer du sein, la surexpression de taux de la protéine HSP60 puisse être un facteur déterminant de cette immunogénité. Murshid et al. (28) ont montré que les protéines chaperonnes Hsps, représente une voie thérapeutique impérative à suivre pour combattre les cancers. La tubuline β , protéine impliqué dans l'architecture du cytosquelette est dans la contraction musculaire a montré par SERPA un rôle immuno-antigénique dans le cancer du sein. Cette protéine intervient dans la motilité cellulaire et dans la structuration de la matrice cytoplasmique. Des études rétrospectives portant sur la détection d'autoanticorps dirigés contre certains antigènes tumoraux ont mis en évidence des autoanticorps anti-tubuline β . Parmi ces travaux, on note celle de Cuij et al. (29) qui ont pu détecter d'autoanticorps anti-tubuline β chez des patients qui souffrent d'une leucémie (29). Des études immuno-protéomiques moyennant l'approche SERPA ont mis en évidence des autoanticorps anti-cytokératine 8 et anti-cytokératine 18 (20). Parmi ces études on note celles de Le Naour F et al. (13) qui ont pu identifier la présence d'autoanticorps anti-cytokératine 8 et anti-cytokératine 18 chez des patientes ayant un cancer hépatique (13). Plusieurs études ont suggéré que la présence d'autoanticorps anti-cytokératine 8 se produira généralement, en réponse aux dommages et à la mort cellulaires qui ont été trouvé aux niveaux sériques des patientes présentant une fibrose pulmonaire, et une autoimmunité d'origine hépatique (13). La production de ces autoanticorps anti-cytokératines 8 et 18 et probablement due à une mal réorganisation des cytokératines qui se produit au cours de l'apoptose. Des autoanticorps anti-cytokératine 18 se sont également déterminés dans les sérums des patients atteints d'un cancer gastrique et chez les patients souffrant d'une fibrose pulmonaire (30). Ces autoanticorps sont dus à la mise en jeu d'une réaction immunitaire vis à vis des constituants du soi (30).

Certains chercheurs montrent que la présence des ces autoanticorps non pathogènes représente un phénomène normal chez un sujet sain (31). Bien que le mécanisme responsable des déclenchements d'une réaction d'autoimmunité contre les cytokératines chez les sujets normaux reste à déterminer, il a été suggéré, que les antigènes immunodominants du cytosquelette puissent être la première cible en cas ou non d'une pathologie maligne. De même, une autre analyse protéomique (SERPA) réalisée par Desmetz et al. (32) ont identifié une protéine du cytosquelette (la cytokératine 18), comme étant des antigènes potentiels du cancer du sein (32). L'approche SERPA, a permis aussi de démontrer la présence d'autoanticorps anti-prohibitine. La prohibitine est un autre antigène qui se trouve capable de déclencher une réaction d'autoimmunité chez les patientes souffrant d'un cancer du sein de type carcinome canalaire infiltrant. C'est une protéine chaperonne qui participe aux défenses antioxydant et participe aux défenses antioxydant (33). C'est une protéine qui est impliquée dans le contrôle de cycle cellulaire, dans la différenciation et jouant le rôle d'une protéine suppresseur de la tumeur. Cette protéine est connue par ses interactions directes avec des protéines qui interviennent dans la régulation du cycle cellulaire, à savoir, la Rb/E2F et la p53 (34). Peu d'études qui ont montré la présence d'autoanticorps contre la prohibitine dans le cadre du cancer du sein, cependant, des études antérieures ont montré la présence des anticorps anti-prohibitine chez de patients atteint d'une leucémie aigue (29). Des autoanticorps qui reconnaissent une protéine suppresseur de tumeur, la P53, ont été mis en évidence dans le sérum de 101 patients ayant une tumeur pulmonaire. Ces autoanticorps anti-P53 sont dus à une altération de la protéine P53 (mutation faux sens) qui est donc responsable de cette malignité. Des mutations ont été également rapportées dans le gène de la prohibitine est qui sont étroitement liée aux cancers du sein et de l'ovaire (34). Bien que les mécanismes responsables de développement de cette immunogénité contre ces antigènes dans le cancer du sein ne soient pas encore élucidés, il est probablement dû à diverse modifications protéiques produites au cours de phénomène apoptotiques (35). Dans ce contexte une autre étude a prouvé que l'anticorps anti-hnRNPB1 (131I-hnRNPB1) permet l'inhibition de la progression tumorale chez les souris (36). D'autres études ont montré que ces protéines (hnRNP) et la prohibitine ouvrent de grands espoirs en médecine dans l'identification d'antigènes vaccinaux dans les domaines thérapeutiques (19), (37) and (38). Sur la même démarche l'approche SERPA a permis d'identifier l'haptoglobine related protein (Hpr) comme étant une autre protéine capable de déclencher une réaction d'autoimmunité (19). Cette protéine est utilisée comme étant un indicateur de la progression de la maladie est de la thérapie antitumorale. Dans une étude réalisée sur le cancer du sein, une immunoréactivité remarquable de l'Hpr a été noté, principalement dans le carcinome invasif plutôt qu'un carcinome mammaire in situ, et que cette réactivité et corrélée avec le phénotype tumoral agressif (39). Epelbaum et al. (40) suggèrent que la synthèse et la sécrétion de l'Hpr par les cellules cancéreuses pourrait être utiles dans le diagnostic de la tumeur, ainsi, ceci peut expliquer au mois un des mécanismes responsables de cette immunoréactivité (40).

Conclusion

La combinaison d'une approche protéomique avec une méthode immunologique, SERPA, a permis finalement, d'identifier de nouvelles protéines à cibler afin d'augmenter l'efficacité de la thérapeutiques anticancéreuses. Cette approche immuno-protéomique SERPA a permis de montrer pour la première fois la présence d'autoanticorps dirigés contre la prohibitine, HSP60, Hpr, β tubuline, et la peroxiredoxine 2 dans le cadre de cancer du sein. Jusqu'à présent, les mécanismes responsables de la synthèse d'autoanticorps sont méconnus. Cette approche immuno-protéomique ouvre maintenant des nouvelles perspectives

prometteuses aussi bien en recherche fondamentale pour une meilleure connaissance des processus biologiques qui régissent la vie d'une cellule, qu'en biomédecine pour l'identification de nouveaux marqueurs liés à la pathologie mammaire. Cependant, d'autres études sont nécessaires sur les tissus tumoraux mammaires pour l'identification de nouveaux antigènes qui sont capables de produire une réponse d'autoimmunité. De même d'autres études sont indispensables, sur un grand nombre des patientes avant et après traitements, qui permet d'évaluer et de cribler les antigènes et/ou les anticorps pour une meilleure utilisation en tant que marqueur de diagnostic et de pronostic des cancers. Ces progrès en biologie moléculaire ont été à l'origine d'une révolution, tant du point de vue de la compréhension des mécanismes oncogénétiques à l'échelle moléculaire, que du développement de nouveaux traitements par un transfert de connaissances de la biologie fondamentale à la clinique.

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Egyptian experience of modified medical thoracoscopy

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Abstract

Background: Thoracoscopy can be performed by a pulmonologist under local/regional anesthesia (medical thoracoscopy) or by a thoracic surgeon under general anesthesia (video-assisted thoracic surgery). The differential diagnosis of pleural disease is often a lengthy process fraught with pitfalls. In pleural malignancies, the diagnostic yield of closed pleural biopsy (CBP) is only 50 to 60% overall, and 20% in malignant mesothelioma (MM). Contrary to thoracocentesis and percutaneous CPB, thoracoscopy permits biopsy with direct visualization. We used a modified technique which is more easy and cheaper in our patients.

Objective and Aim of the Study: The aim of this study to evaluate the use of modified medical thoracoscopy in undiagnosed pleural effusion.

Methods: An informed consent was taken from patients before the beginning of the study. For each patient, one should analyze the following: (1) detailed medical history, including smoking habits, exposure to asbestos, and the personal history of cancer; (2) chest radiographs and CTs, in order to assess pleural effusion when existing; and (3) the results of available closed pleural biopsies (CPBs). Also, bleeding and clotting profile should be done preoperative. Prior to the procedure, pleural effusion was fully drained in the endoscopy suite. Thoracoscopy was done under local/regional anesthesia with spontaneous breathing and mild sedation (midazolam) by an experienced pulmonologist in the thorascopic room. Patients were placed in lateral decubitus position, with the involved side upward. After skin sterilization and local generous 2% xylocaine anaesthesia, a 2-4 cm incision was done and blunt dissection was used to enter the pleural space between the third and sixth intercostal space, along the midaxillary line. The site of introduction is usually determined using ultrasonography of the most dependant area of the effusion. A 40F silicone tube 10 cm long was inserted into the incision. A sterilized fiberoptic bronchoscope signed for thoracoscopy only was inserted, and the pleural cavity was inspected through 40F silicone tube. The parietal, visceral, and diaphragmatic pleura were successively inspected, together with the mediastinal vessels and lymph nodes. Biopsies were performed under direct visual control in all suspect areas, systematically in several parts of the parietal pleura, and sometimes in the visceral pleura and will be sent for pathological examination. An intercostal tube was inserted before wound closure to evacuate air and fluid. Chest radiographs were routinely, immediately after the procedure and daily thereafter until chest tube removal. When indicated, pleurodesis was performed secondarily by brushing, tetracycline installation (or Bovodine) in the pleural space.

Results: Twenty two patients were admitted to chest department with first pleural

tapping undiagnostic. The age range from 28 to 70 years old, with mean of 50.8 ± 10.5 years. Fourteen patients (63.6%) were males, and 8 patients (36.4%) were females. Smokers were 13 (59.1%), and 9 (40.9%) were non-smokers. The effusion was right sided in 12 (54.5%) and left sided in 10 (45.5%). The pathology was diagnostic in 18 (81.8%) and non-specific in 4 (18.1%). We found malignant mesothelioma (18.2%), non-specific inflammation (18.2%), tuberculosis (13.6%), adenocarcinoma (13.6%), metastasis from primary breast cancer (13.6%), poorly differentiated carcinoma (9.1%), anaplastic carcinoma (4.5%), non-Hodgkin lymphoma (4.5%), and small cell carcinoma (4.5%).

Conclusion: Modified medical thoracoscopy is an easy cheap technique with no complications. We recommend this technique where resources and surgical thoracoscopy is not available or expensive.

Introduction

Thoracoscopy is not a new technique; H.C. Jacobeus, the Swedish internist, was the first to perform thoracoscopy in 1910, as a diagnostic procedure for exudative pleuritis. H.C. Jacobeus published the first series of thoracoscopy cases in 1921, describing the value of thoracoscopy in the diagnosis of tuberculous and malignant effusions. However, in the following decades, thoracoscopy was used mainly as a therapeutic tool for adhesiolysis in patients with tuberculosis (TB), in order to obtain a "therapeutic" pneumothorax. After the decline of thorascopic interventions as a treatment for TB, some centres in continental Europe continued to use thoracoscopy as a diagnostic and therapeutic tool in other disorders, such as pneumothorax and pleural effusion (1). In the early 1960s, thoracoscopy was used, mainly by pneumologists in Europe, on a much broader basis for the diagnosis of many pleuropulmonary diseases (2). Due to technical improvements, thoracoscopy was rediscovered by thoracic surgeons at the beginning of this decade, and renamed "surgical" thoracoscopy, better known as video-assisted thoracic surgery (VATS), requiring general anaesthesia with selective endobronchial intubation, disposable equipment, and at least three points of entry (3-4).

Medical thoracoscopy is a minimally invasive procedure performed by the pneumologist in an endoscopy suite, is much less invasive requiring only local anaesthesia with conscious sedation and only one or two points of entry. It also allows for basic diagnostic (undiagnosed pleural fluid or pleural thickening) and therapeutic procedures (pleurodesis) to be performed safely and distinct from video-

assisted thoracoscopic surgery, an invasive procedure that uses sophisticated access platform and multiple ports for separate viewing and working instruments (5). In Europe, thoracoscopy is intrinsic in the training programme of pneumology (6). In the USA, according to a national survey in 1994, only 5% of all pulmonologists were applying medical thoracoscopy (2).

The main indications of medical thoracoscopy are the diagnosis and treatment of pleural effusions and pneumothorax. In pleural effusions medical thoracoscopy provides the proof or exclusion of malignancy and tuberculosis with an accuracy approaching 100%. As a staging procedure it helps determine the aetiology and extent, and possibly, prognosis of malignant effusions as well as treatment strategies. The insufflation of talc powder during thoracoscopy is the best conservative method of pleurodesis in malignant and recurrent benign effusions, including chylothorax. Medical thoracoscopy has proved also to be successful in the management of empyema and of spontaneous pneumothorax. In the future, it may become even more popular once more respiratory physicians are trained in the procedure (2).

Patients and Methods

An informed consent was taken from patients before the beginning of the study. For each patient, one should analyze the following: (1) detailed medical history, including smoking habits, exposure to asbestos, and the personal history of cancer; (2) chest radiographs and CTs, in order to assess pleural effusion when existing; and (3) the results of available closed pleural biopsies (CPBs). Also, bleeding and clotting profile should be done preoperative. Prior to the procedure, pleural effusion was fully drained in the endoscopy suite. The medical thoracoscopy usually is performed by a pulmonary physician and an assistant in an endoscopy suite with one or two trained nurses and an anesthesiologist. Thoracoscopy was done under local/regional anesthesia with spontaneous breathing and mild sedation (midazolam) by an experienced pulmonologist in the thoracoscopic room. The patient's BP, pulse rate, and oxygen saturation to be monitored continuously. Supplemental oxygen often given to the patients to maintain oxygen saturation (7). Patients were placed in lateral decubitus position, with the involved side upward. After skin sterilization and local generous 2% xylocaine anaesthesia, a 2-4 cm incision (Fig.1) was done and blunt dissection was used to enter the pleural space between the third and sixth intercostal space, along the midaxillary line. The site of introduction is usually determined using ultrasonography of the most dependant area of the effusion. A 40F silicone tube 10 cm long was inserted into the incision (Fig.2). A sterilized fiberoptic bronchoscope signed for thoracoscopy only was inserted, and the pleural cavity was inspected through 40F silicone tube (Fig.3). The parietal, visceral, and diaphragmatic pleura were successively inspected, together with the mediastinal vessels and lymph nodes. Biopsies were performed under direct visual control in all suspect areas, systematically in several parts of the parietal pleura, and sometimes in the visceral pleura and will be sent for pathological examination. An intercostal tube was inserted before wound closure to evacuate air and fluid (Fig.4). Chest radiographs were routinely obtained, immediately after the procedure and daily thereafter until chest tube removal. When indicated, pleurodesis was performed secondarily by brushing, tetracycline installation (or Bovodine) in the pleural space.



Fig 1: The typical skin incision



Fig 2: A 40F silicone tube 10 cm long was inserted into the incision

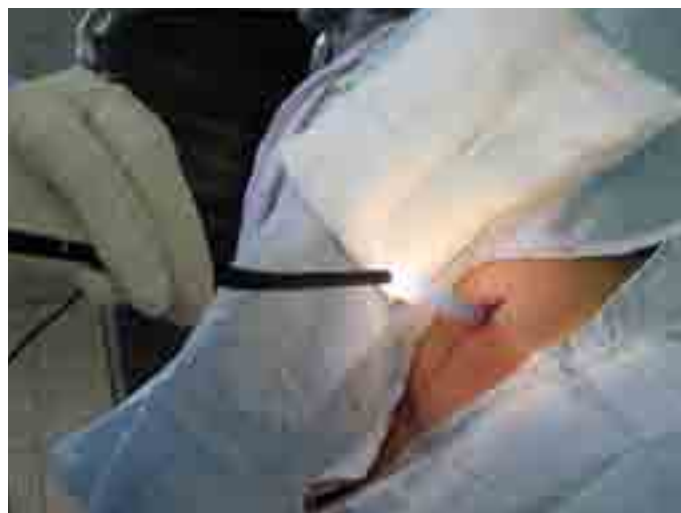


Fig 3: A sterilized fiberoptic bronchoscope inserted, and the pleural cavity inspected through the 40F silicone tube



Fig 4: An intercostal tube was inserted before wound closure

Results

Twenty two patients were admitted to chest department with first pleural tapping undiagnostic. The age range from 28 to 70 years old, with mean of 50.8 ± 10.5 years. Fourteen patients (63.6%) were males, and 8 patients (36.4%) were females. Smokers were 13 (59.1%), and 9 (40.9%) were non-smokers. The effusion was right sided in 12 (54.5%) and left sided in 10 (45.5%). The pathology was diagnostic in 18 (81.8%) and non-specific in 4 (18.1%). We found malignant mesothelioma (Fig.5) in (18.2%), non-specific inflammation (Fig.6) in (18.2%), tuberculosis (Fig.7) in (13.6%), adenocarcinoma (Fig.8) in (13.6%), metastasis from primary breast cancer (13.6%), poorly differentiated carcinoma (9.1%), anaplastic carcinoma (4.5%), non-Hodjkin lymphoma (Fig.9) in (4.5%), and small cell carcinoma (4.5%).

Pleural biopsy specimens were obtained in all the 22 cases, and all specimens were deemed to be of satisfactory quality. A definitive histologic diagnosis was made in 18 of the 22 patients who underwent thoracoscopy for evaluation of an unexplained exudative effusion, and malignancy was discovered in almost two third of the cases. Mean duration of chest tube drainage was 2.5 ± 1.4 days postprocedure

Medical thoracoscopy was a safe and easy procedure in our patients. Procedure-related mortality was 0.00%. Potential adverse events that occurred during or after the procedure included mild bleeding in 1 (4.5%) patient, persistent pneumothorax in 2 (9.1%) patients, sinus tachycardia 2 (9.1%) patients, low-grade fever in 4 (18.2%) patients, there were no complications related to anesthesia, no respiratory failure occurred, wound infections in 2 (9.1%) patients, empyema in 1 (4.5%) patient, and severe pain in 3 (13.6%) patients.



Fig 5-A: Thoracoscopic view showing diffuse infiltration of mesothelioma

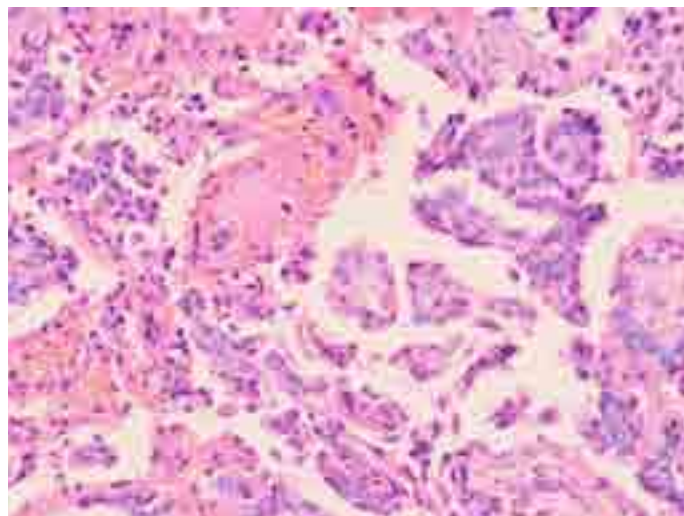


Fig 5-B: Pathological examination reveals tumor tissue composed of regular cells with regular nuclei, scanty cytoplasm arranged in acini and papillary structures a classic features of mesothelioma (H&E Stain x 200)



Fig 6-A: Thoracoscopic view showing non-specific inflammation

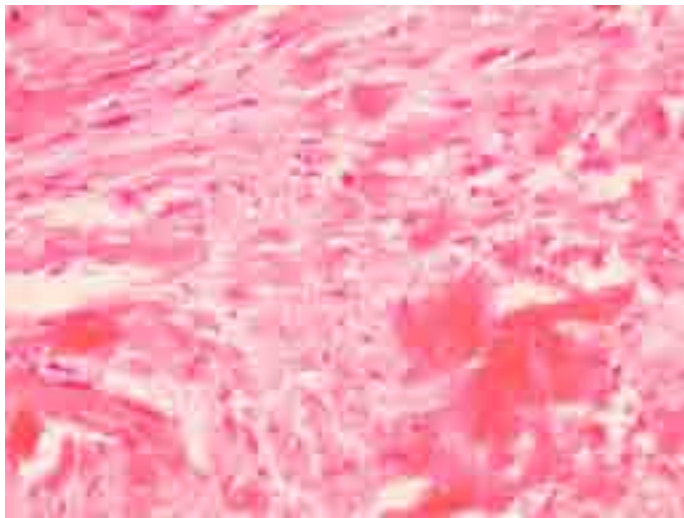


Fig 6-B: Pathological examination reveals markedly thickened pleural membranes entangling few scattered mononuclear inflammatory cells (H&E Stain x 400)



Fig 8-A: Thoracoscopic view showing nodular infiltrations of adenocarcinoma



Fig 7-A: Thoracoscopic view showing diffusely inflamed thickened pleural surfaces with multiple adhesions

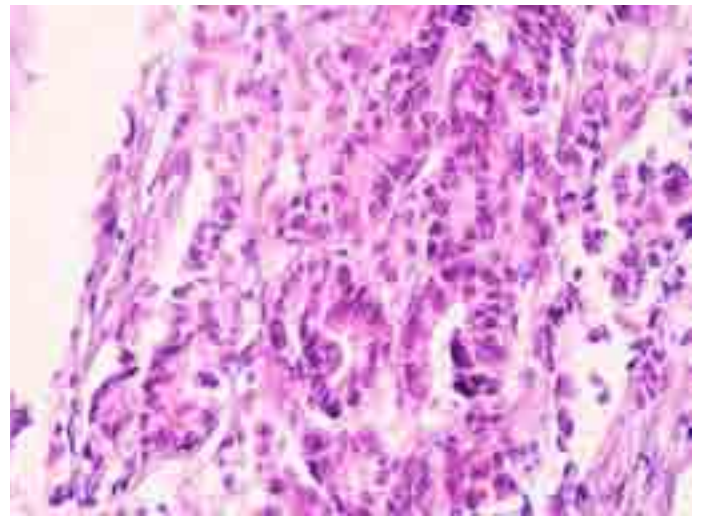


Fig 8 B: Pathological examination reveals metastatic deposits of well organized glandular cells with nuclear atypia of metastatic well differentiated adenocarcinoma (H&E Stain x 400)

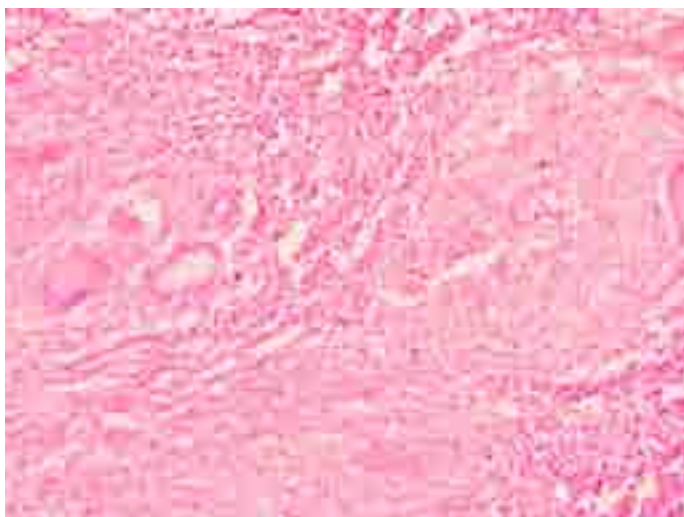


Fig 7-B: Pathological examination reveals granulomatous lesion composed of langehans giant cells surrounded by epithelioid cells with caseous necrosis (H&E Stain x 200)



Fig 9-A: Thoracoscopic view showing nodular diffuse infiltrations of non-Hodgkin lymphoma

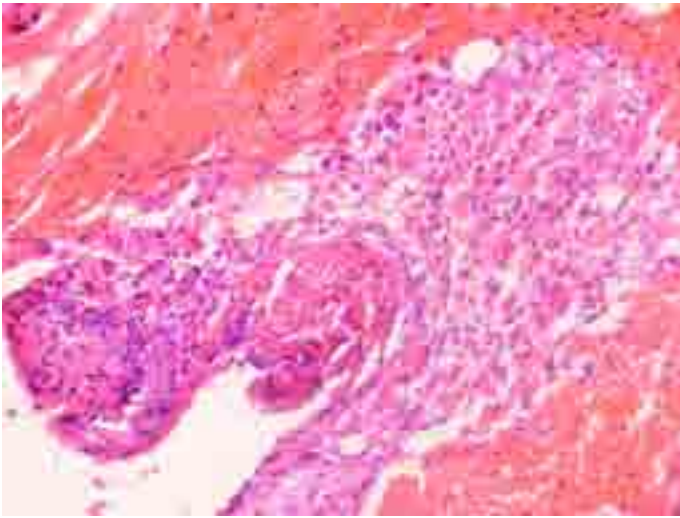


Fig 9-B: Pathological examination of non-Hodgkin lymphoma showing small malignant lymphoid cells (H&E Stain x 400)

Discussion

We use a modified technique for medical thoracoscopy which is an easy technique and cheaper for medical patients. We found this technique was diagnostic in 18 (81.8%) and non-specific in 4 (18.1%). The pathology was malignant mesothelioma in (18.2%) of patients, non-specific inflammation in (18.2%) of patients, tuberculosis in (13.6%) of patients, adenocarcinoma (13.6%) of patients, metastasis from primary breast cancer in (13.6%) of patients, poorly differentiated carcinoma in (9.1%) of patients, anaplastic carcinoma in (4.5%) of patients, non-Hodgkin lymphoma in (4.5%) of patients, and small cell carcinoma in (4.5%) of patients.

Davidson AC, et al (8); had used the fiberoptic bronchoscope for inspection of the pleural space and compared it with rigid thoracoscope in 1988. The practicality of physicians performing thoracoscopy for diagnostic purposes was assessed in 30 patients with pleural effusions of unknown cause. A rigid thoracoscope was compared with a fiberoptic bronchoscope used as a flexible thoracoscope and the diagnostic adequacy of biopsy specimens obtained with the two instruments assessed. The two instruments were inserted by a physician in the bronchoscopy suite using local anaesthesia. The procedure proved safe, acceptable, and diagnostically effective. The rigid thoracoscope proved a more satisfactory instrument but the fiberoptic bronchoscope, with minor adaptations, may be used for thoracoscopy (8).

One method which essentially used local anaesthesia and one opening for the scope and instrument. This method was described by Kolschmann et al (7), who used Lidocaine 2% for local anaesthesia, and sedation was achieved by a combination of midazolam and fentanyl. They used a 6.5-mm thoracoscope (0° and 30°; Karl Storz; Tuttlingen, Germany) with a single 7-mm trocar. After complete aspiration of all of the remaining fluid, a thorough inspection of the pleural surface was made. The adhesions were taken down with the biopsy forceps, if possible. Biopsy specimens were made for histopathologic examination, if necessary. Under visual control, an average of 8 g of sterile asbestos-free talc (Steritalc; Novatech; France) were distributed onto the pleural surface for pleurodesis. After removal of the thoracoscope, a thoracostomy tube (24 Charrière) was inserted. Suction (-20 cm H₂O) was started after 1 h, and the chest tube was left in place until < 100 mL of fluid was drained in 24 h. Chest radiography was performed the same day after the procedure and before discharge (7).

Flexible bronchoscopes have also been used by other authors, which in comparison with rigid thoroscopes have several disadvantages, in particular the less adequate orientation within the pleural cavity and the smaller biopsies (9). Most authors use flexible instruments only because rigid instruments are not available or appear dangerous: some authors believe that local anaesthesia is not adequate (10).

Ernst et al (11); described their experience with the use of a novel endoscope that is similar in design to a commonly used bronchoscope. This pleuroscope interfaces with existing processors and light sources that are routinely employed for flexible bronchoscopy and, therefore, are available in most endoscopy units. The instrument used was a prototype semirigid pleuroscope the outer diameter of the shaft is 7.0 mm. The length of the insertion portion is 27 cm, which consists of a proximal rigid portion (22 cm) and a bendable distal end (5 cm). The tip is movable in one plane with the help of a lever on the handle, which is similar to a conventional flexible bronchoscope. A 2.8-mm single working channel accommodates the biopsy forceps and other instruments (11). The most common indications were for pleurodesis of a malignant pleural effusion (53%) or for evaluation of an exudative effusion of unknown etiology (44%). Pleural biopsy specimens were obtained in 13 cases, and all specimens were deemed to be of satisfactory quality. A definitive histologic diagnosis was made in 4 of the 14 patients who underwent pleuroscopy for evaluation of an unexplained exudative effusion, and malignancy was discovered in all 4. Pleural biopsies were performed in 13 patients, and talc pleurodesis procedures were performed in 25 patients. Mean duration of chest tube drainage was 2.9 ± 1.8 days postprocedure. There were no complications (11).

Tassi et al (12); evaluated minithoracoscopy using 3-mm instrumentation for diagnosis of pleural effusions. The basic components for minithoracoscopy are two 3.8-mm trocars, one 3.3-mm telescope, and one 3.0-mm biopsy forceps. The key instrument is the telescope (Karl Storz Endoskope; Karl Storz; Tuttlingen, Germany), which is 25 mm in length and has viewing angles of 0° and 45°. Indication was later extended to larger nonoculated effusions that could have been examined using conventional thoracoscopy. A total of 30 patients were studied, including 12 patients with nonoculated effusions of undetermined etiology, 17 patients with loculated effusions, and 1 patient with bilateral effusion. In two patients with mesothelioma, lung biopsy samples obtained by minithoracoscopy allowed diagnosis of invasion from the visceral pleura. In the remaining patient, the sample was not interpretable due to coagulation-related artifacts (12). Minithoracoscopy provided high diagnostic yield (93.4%). Visualization using minithoracoscopy instrumentation was equal to that obtained using conventional thoracoscopy instrumentation. Tolerance and cosmetic results were good. Minithoracoscopy is safe and effective for routine diagnostic applications (12).

Potential advantages of modified medical thoracoscopy over more conventional techniques include certainty of representative tissue for diagnosis, reduced requirements for postoperative analgesia, shorter hospital stays, and a shorter duration of chest tube drainage compared with thoracotomy (13). Also, it was studied to decrease procedure-related costs by employing reusable instruments. Additional studies are necessary to determine ideal settings for thoracoscopic intervention and to evaluate current perceptions regarding thoracoscopic practice (14).

Conclusion

Modified medical thoracoscopy is an easy and safe technique with less complications. It is less expensive than rigid thoracoscopy, and thoracotomy in many diagnostic and therapeutic indications. The instruments needed are so simple

and re-usable. The outcome is safe and results are satisfactory and conclusive. We recommend this technique where resources and surgical thoracoscopy is not available or expensive especially in developing countries and where resources are limited with equal and promising results as rigid thoracoscopy.

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Efficacy and tolerability of cisplatin and adriamycin as combination chemotherapy for Malignant pleural mesothelioma

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Abstract

Pleural mesothelioma is a locally invasive and aggressive tumor with a poor prognosis. Its incidence is increasing throughout most of the world. Therefore, effective systemic chemotherapy for pleural mesothelioma is clearly needed. This study is prospective phase II study to evaluate the efficacy and tolerability of cisplatin and adriamycin as combination chemotherapy for malignant pleural mesothelioma.

Patients & Methods: Between December 2005 and March 2007, 30 patients were registered. Eligibility criteria included Karnofsky performance status ≥ 60 and no prior chemotherapy. Of 30 patients entered, 25 were included, 17 of them have epithelial cell type and 8 with sarcomatous or mixed cell type. Patients received a total of six cycles of adriamycin (50mg/m²) and Cisplatin (80mg/m²) chemotherapy regimen.

Results: Overall response rate was 16%. Stabilization rate including partial response and no change was 56%. Median response duration was 8 months (range 4 – 12 months). Median overall survival was 12 months (range 7 – 16 months). Performance status was considered a potential prognostic factor for overall survival ($p = 0.04$). The main toxicities were nausea and vomiting, neutropenia, thrombocytopenia and alopecia. No toxic death was documented.

Conclusion: Combination of cisplatin and adriamycin is active well tolerated chemotherapy with reasonable toxicity for patients with pleural mesothelioma. However, it does not demonstrate superior activity to other active regimens in this disease.

Introduction

Pleural mesothelioma is a tumor derived from the mesothelium covering the surface of pleural membranes or from undifferentiated mesenchymal cells in connective tissue under the membranes. It is a locally invasive and aggressive tumor with a poor prognosis and a median survival time of ≈ 9 –16 months (1).

Pleural mesothelioma is known to be linked to asbestos exposure, and the incidence of this tumor is expected to increase in the next 10–20 years according to an estimation of asbestos consumption in the world. Its incidence is increasing throughout most of the world, and it is predicted that it will rise in the next 10 to 20 years (2).

Surgical resection offers local control of the tumor but its effect on survival remains unclear. In addition, application of radiation therapy is limited because of the diffuse extension of tumor spread. Whole lung radiotherapy has not demonstrated its efficacy in controlling the disease (3). Finally, numerous chemotherapeutic agents have been tested in phase II studies (3). Response rate does not exceed 20% for the majority of the investigated regimens. In narrative reviews, agents such as cisplatin, doxorubicin, high dose methotrexate, alkylating agents or mitomycin C appear potentially active (4,5).

A systematic review was conducted, recently updated, of the fully published literature with a methodological quality assessment of the studies relative to chemotherapy or immunotherapy in malignant mesothelioma (6,7). They aggregated, for similar quality studies, response rates to identify the more active chemotherapeutic drugs and regimens, in order to use the obtained information for future trials. Ninety-five studies (100 treatment arms) were eligible for the systematic review. Overall, they concluded that cisplatin-based chemotherapy, when associated with doxorubicin, etoposide or gemcitabine, presented with significantly better response rate than other chemotherapy regimens and that polychemotherapy was superior to single-agent therapy (7).

Regimens applied to lung cancer such as platinum-containing chemotherapy have been used for MPM in Japan; however, the efficacy outcomes of these therapies are not satisfactory (8).

The European Lung Cancer Working Party (ELCWP) decided to conduct a prospective phase II study assessing the activity of combination chemotherapy that showed that cisplatin plus epirubicin appears as an effective regimen in malignant mesothelioma, with a favorable toxicity profile. However, it does not demonstrate superior activity to other active regimens in this disease (9).

Therefore, effective systemic chemotherapy for MPM is clearly needed.

Recently, Vogelzang et al. demonstrated in a randomized phase III trial that a combination of cisplatin and pemetrexed (Alimta®) was superior to single agent cisplatin both for response rate and survival (10).

Aim of work

- Evaluate the efficacy and tolerability of cisplatin and adriamycin as combination chemotherapy for malignant pleural mesothelioma .
- Follow up for the toxicity profile of cisplatin and adriamycin as combination chemotherapy .
- Assess the overall response and quality of life for malignant pleural mesothelioma patients receiving combination chemotherapy .

Patient and methods

This study was conducted from December 2005 to March 2007 in Kaser El-Aini Center of Oncology and Nuclear Medicine.

Eligibility criteria

Patients had to present with previously untreated histologically confirmed mesothelioma . An assessable or measurable lesion has to be present. Patients should not have prior history of malignancy or previous chemotherapy intake . Other eligibility criteria included Karnofsky performance status ≥ 60 , good renal function (serum creatinine level ≤ 1.5 mg/dl and creatinine clearance ≥ 90 ml/min), hepatic function (serum bilirubin level ≤ 1.1 mg/dl , aspartate and alanine transferase levels up to 3 times the upper limit of normal) and haematological profile (neutrophil count ≥ 2000 mm⁻³ , hemoglobin > 9 g/dL and platelet count $\geq 100,000$ mm⁻³) .

Exclusion criteria

Patients presenting with recent (<3 months before the date of treatment) myocardial infarction, congestive heart failure or cardiac arrhythmia requiring medical treatment, uncontrolled infectious disease or other serious medical or psychiatric illness precluding adherence to the study protocol were excluded.

Investigations

Initial work-up included clinical evaluation completed by weight, height, surface area and record of Karnofsky performance status, complete blood sampling including evaluation of creatinine clearance , chest X-ray and CT scan (in case of pleurodesis, chest CT scan must be repeated before administration of the first course of chemotherapy), abdominal ultrasound, isotopic or echographic left ventricular assessment and assessment of hearing by audiometry .

Blood sampling were performed before each cycle . An evaluation after each three courses of chemotherapy was performed with the same tests as during the initial work-up . This assessment was repeated every three cycles. After treatment completion, patients were followed every 2 months with clinical evaluation. Chest X-ray and biological tests if needed .

Treatment

The chemotherapy regimen consisted in the combination of cisplatin and adriamycin. Adriamycin (50mg/m²) was given as a 30 min intravenous infusion over 2 days (D1-2) , just before cisplatin administration. Cisplatin (80mg/m²) was administered over 120 min in 500 ml NaCl 0.9%, after pre-hydration with 1.5 liters of 0.9% NaCl for 6 h and followed by a mannitol-induced diuresis over 3 days (D1-3). The recommended antiemetic regimen was a combination of dexamethasone and granisetron or zofran .

Courses were repeated every 3—4 weeks, as soon as haematological (neutrophils > 1500 mm⁻³ , hemoglobin > 9 g/dL and platelets $> 100,000$ mm⁻³) and renal (creatinine < 1.1 mg/dl and creatinine clearance ≥ 90 ml/min) functions have recovered.

The patient went off treatment if myelosuppression persisted on day 36 , progressive impairment of cardiac function as evaluated by left ventricular assessment or hearing function as evaluated by audiometry .

Criteria of evaluation

Patients were considered assessable if three courses of chemotherapy were completed. Patients with early progression or death prior to evaluation due to malignant disease or toxicity and treatment cessation due to toxicity were considered as treatment failures and incorporated in the evaluable patients.

Duration of response was calculated from the day of registration until the date of first observation of progressive disease. Survival was dated from the day of registration.

Response was evaluated by a complete restaging after each three courses. Complete remission was defined as the disappearance of all signs of disease. Partial response was defined as a 50% or greater decrease in the lesions for at least 3 weeks in the absence of progressive disease or occurrence of new lesions elsewhere. Progression was considered to be an increase of greater than 25% in lesion or the appearance of a new lesion, irrespective of response elsewhere. All other circumstances were classified as no change. WHO criteria were used to assess toxicity.

Statistical methods

Kaplan Meier method was used for estimating overall survival, and relapse free survival. P-value is considered significant at 0.05 level. Numerical data were described in terms of means and medians for central tendency and standard deviation and range, minimum and maximum for dispersion.

Results

Between December 2005 and March 2007 , 30 patients were registered . Five patients were deemed ineligible due to poor performance status and were offered supportive treatment only .

Characteristics of 25 eligible patients are shown in table 1 . The majority of patients were males (60%) , had a good performance status of 70 or more on Karnofsky scale (72%) and presented with epithelial histology subtype (68%) . Most of the patients had assessable disease (84%) and most of them presented with platelet count $\leq 400,000$ / μ L (88%) .

Table 1: Characteristics of 25 eligible patients with malignant mesothelioma

Eligible	25
Gender	
Male	16
Female	9

Median age (range)	48 (28-65)
Performance status	
60	7
70	15
80	3
Histology	
Epithelial	17
Sarcomatoid	5
Biphasic	3
Disease assessment	
Measurable	4
Assessable	21
Platelet count (μL)	
> 350.000	3
≤ 350.000	22

Overall 111 chemotherapy cycles were administrated along the study, ranging from one to six per patient. Fourteen patients completed six cycles, eight patients received three cycles and three patients received one cycle of chemotherapy cisplatin and adriamycine. After one cycle three patients had disease progression and after three cycles eight more patients had disease progression and shifted to another line of treatment. After six cycles of treatment four patients had partial response and ten patient had stable disease.

Overall response rate was 16%. Stabilization rate including partial response and no change was 56%. Median response duration was 8 months (range 4 – 12 months). Median overall survival was 12 months (range 7 – 16 months).

Table 2: Response frequencies

Response	No	%
Complete	0	0
Partial	4	16%
Stable	10	40%
Progression	11	44%

As expected, the main toxicities were nausea and vomiting, neutropenia, thrombocytopenia and alopecia. No toxic death was documented. Highest toxicity per patient during the whole course of treatment is reported in table 3 .

Table 3: Highest toxicity during the whole treatment among patients at least receiving three cycles of chemotherapy (n=25)

	0	I	II	III	IV
Neutropenia	1	8	8	6	2
Thrombocytopenia	9	4	7	4	1
Nausea	2	8	9	6	-
Diarrhea	20	2	3	-	-
Stomatitis	14	2	5	4	-
Infection	20	2	3	-	-
Neurologic	19	2	4	-	-
Cardiac	25	-	-	-	-
Hearing loss	-	14	11	-	-
Nephrotoxicity	13	5	7	-	-

A prognostic factors analysis for survival was performed . In univariate analysis,

age ($p = 0.71$) , gender ($p = 0.61$) , histology (epithelial versus other ; $p = 0.08$) , and platelet count ($p = 0.06$) were not considered as significant factors. Performance status ($p = 0.04$) was considered as potential prognostic factor for overall survival.

Discussion

The reasons for a lack of consensus in the management of mesothelioma are partly historical, partly related to the unique biology of the tumor and partly due to failure so far to find any single treatment or treatment combination which offers more than short-term tumor suppression in most patients. Historically, the very existence of a primary tumor of the pleura was disputed in the first half of this century (12) and, even when it became accepted as a distinct entity with a clear relationship to asbestos exposure in many patients (13), difficulties were still encountered in distinguishing the tumor from adenocarcinoma in many instances (14).

Until more sophisticated immunocytochemical techniques were developed to distinguish between epithelial mesothelioma an adenocarcinoma, there is no doubt that many cases treated as mesothelioma were, in fact, cases of secondary adenocarcinoma. This added to the confusion in the interpretation of the results of treatment (15).

The biological behavior of mesothelioma is distinct from that of other solid tumors in that mesothelioma tends to grow in a sheet-like fashion, covering the surface of the parietal and then the visceral pleura; it shows little tendency to invade structures deep into the pleura in the early course of the disease, unless the pleura is breached by needles or tubes, when it will spread readily along needle or tube tracks (16).

Part of the explanation for this unusual behavior appears to lie in the relative lack of proteases in comparison to other solid tumors (17). In many instances the tumor appears to begin in a multifocal fashion resulting in scattered deposits of tumor with normal pleura intervening, suggesting either that a field change has occurred throughout the pleura, or that the tumor has metastasized locally within the pleural cavity (18).

Because the tumor is either broadly extensive on the pleural surface or multifocal at the time of detection, it does not lend itself to localized surgical excision. Surgical treatment aimed at complete resection therefore has to be much more extensive and is only suitable for a minority of patients (15) .

Currently, the treatment of malignant mesothelioma remains controversial. No single therapy has demonstrated to have an impact on survival in randomized trials. In a randomized feasibility study, chemotherapy allowed a better symptom control than best supportive care alone (11).

However, malignant mesothelioma is considered by most oncology physicians as a poorly chemotherapy sensitive tumor and there is no universally accepted standard chemotherapy. In a previous review (7), it was found that cisplatin plus doxorubicin was one of the most active chemotherapy regimens with an overall RR of 28.5% (9) .

In the study , cisplatin and adriamycine was associated with overall response rate was 16% , stabilization rate including partial response and no change was 56% and median response duration was 8 months (range 4 – 12 months) with median survival of 12 months (range 7 – 16 months) that is comparable to other

chemotherapy regimens used to treat pleural mesothelioma (3 , 4 , 5 and 9) . Low response rate and short duration of median survival can be attributed to small sample size and short duration of follow up due to non coherence of patients on follow up schedules due to some financial and social factors .

The toxicity profile is comparable to the study done by Berghmans et al . 2005 (9) using cisplatin and epirubicin combination. The main toxicities were nausea and vomiting , neutropenia , thrombocytopenia and alopecia . No sever renal toxicity was noted that necessitate discontinue of treatment and also no persistent hearing was noted . No gross cardiac toxicity was noted .

The prognostic role of multiple factors on survival has been investigated in malignant mesothelioma in numerous studies. Stage, performance status, histology and WBC count were commonly reported as having a significant impact on survival. In the study, only performance status was noted as a potential prognostic factor for overall survival mostly due to low sample size,

In 1998, the European Organization for Research and Treatment of Cancer (EORTC) identified several prognostic variables for the course of the disease. In a multivariate analysis of the EORTC, poor prognosis was associated with high WBC count , poor performance status, low hemoglobin level, probable/ possible histologic diagnosis of mesothelioma and having sarcomatous histologic subtype (19).

Likewise, the Cancer and Leukemia Group B (CALGB) analyzed several pretreatment factors pooled from seven phase II studies that were predictive of poor survival and defined six prognostic groups. Poor prognosis was seen in patients with the following criteria: age older than 75 years, poor performance status, chest pain, dyspnoea, weight loss, high white blood cell count, elevated platelet count, low haemoglobin, elevated serum lactate dehydrogenase levels, pleural effusion and nonepithelial histology (20).

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Gemcitabine/Cisplatin in the treatment of metastatic breast cancer patients pretreated with anthracyclines

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Abstract

Purpose: A phase II prospective study to evaluate efficacy and tolerability of gemcitabine and cisplatin as a first line combination chemotherapy in patients with metastatic breast cancer (MBC) pretreated with anthracyclines in their adjuvant setting.

Patients & Methods: Patients were assigned to receive gemcitabine 1250mg/m² on days 1 & 8 plus cisplatin 75 mg/m² day 1, repeated every 3 weeks (for 6-8 cycles).

Results: The study included 40 female patients with MBC and took place during the period from December 2006 to June 2009 with a median follow up period of 12 months (range 3-24 ms). The overall objective response rate was 57.9%. The median duration of response was 9.5 ms (95% CI, 8.07 to 11.83 ms) and the median time to disease progression was 12.5 ms (95% CI, 10.85 to 14.43 ms). The estimated median survival was 22 ms (95% CI, 16.34 to 27.66 ms). The 1 and 2 years survival probabilities were 68.52% and 31%, respectively. The study regimen proved to be quite tolerable with the main hematological toxicities of this protocol were grade III anemia in 10% of patients, grade III/IV neutropenia in 20% of patients and grade III/IV thrombocytopenia in 17.5% of patients. There were no grade IV non hematological toxicity observed within the study and the only grade III non hematological toxicities recorded in the study were grade III nausea in 25% of patients, grade III vomiting in 17.5% of patients, grade III fatigue in 15% of patients and grade III renal toxicity in 2.5% of patients.

Conclusion: Gemcitabine / cisplatin combination was both effective and tolerable as first-line therapy in MBC pretreated with anthracyclines. However, initiation of larger phase III studies comparing gemcitabine cisplatin combination directly with other chemotherapy combinations in MBC patients pretreated with anthracyclines is recommended.

Introduction

Metastatic breast cancer (MBC) represents 10% of newly diagnosed breast cancer patients. Moreover, a substantial portion of the early and localized breast cancer patients will metastasize sooner or later along the course of their disease.¹

In spite of major advances in screening, surgery, radiation, endocrine therapy and chemotherapy of patients with early stage breast cancer, modest progress has been achieved in improving survival for women with metastases. The median survival for patients with metastases remains between 18 and 24 months.²

The primary objective of different lines of treatment for MBC patients is palliation, not cure. Treatment aims at controlling the progression of the patient's disease, improve quality of life (QOL) and improve or eliminate tumor-related symptoms.³

Endocrine therapy of MBC patients achieves objective response in more than 30% of patients that generally lasts an average of 1 year which is usually several months longer than response to chemotherapy when used in the same setting.⁴

However, with the overall response rate (RR) to chemotherapy being higher than that to endocrine therapy, patients with rapidly progressive tumors or major tumor-related symptoms should be considered for chemotherapy.⁵

Anthracycline-based regimens if not previously used in the adjuvant (adj.) setting are the first chemotherapy option in treatment of MBC, it can usually achieve an overall RR around 65% and time to progression (TTP) averaging around 12 months.⁶

Taxanes were used either as single agent or in combination in MBC. Single agent taxanes were found to be active in both doxorubicin-naive and doxorubicin-refractory MBC (RRs averaging between 25% to 55%) with a better toxicity profile in comparison to taxane combinations regimens but it failed to achieve survival impact.⁷

Taxanes were used in combination with a wide spectrum of chemotherapeutics with better RRs but on the expense of increase of the side effects percentages that could compromise the patients' QOL. In addition these RRs did not translate into a significant survival advantage added to the fact that MBC remains an incurable disease.⁸

Financial, reducing toxicities and QOL issues led to the usage of non-taxane active drugs like vinorelbine, capecitabine and gemcitabine as available options for MBC treatment.⁸

Gemcitabine "a pyrimidine antimetabolite" was found to be both effective and safe in combination with different drugs in both locally advanced and MBC.⁹

Cisplatin, one of the classical chemotherapeutics had been used repeatedly as

an important agent in many active chemotherapeutic doublets in most cancer subtypes including MBC with a considerable effectiveness and good tolerance.¹⁰ Drug resistance to cisplatin may be overcome when used in combination with gemcitabine.¹¹

The benefit gained from this combination would be maximized by using it as a first line therapy instead of second line or salvage therapy in poly treated patients where more side effects and less probability of RRs would be expected.¹²

In 2006, the results of Fuentes and colleagues who enrolled 46 patients with MBC to receive gemcitabine and cisplatin as first line therapy showed that 17% of patients achieved complete response “CR” and 64% achieved partial response “PR” with an overall RR of 81%. From this they concluded that gemcitabine plus cisplatin is a highly effective and safe first line treatment for patients with MBC and the TTP of 14.9 ms compares favorably with other standard treatments as anthracyclines and taxanes.¹³

Thus the current phase II prospective study was conducted to evaluate the efficacy & safety of gemcitabine and cisplatin combination chemotherapy as first line chemotherapy in MBC patients previously treated with adj. anthracyclines in terms of degree of response, time to disease progression and treatment related toxicities.

Patients and methods

Eligibility criteria

Patients entered onto this study were required to have histological or cytological proven MBC with at least one bi-dimensionally measurable lesion, age ≥ 18 years and ≤ 65 years, performance status of 0-2 on the ECOG performance status scale 4, estimated life expectancy of at least 3 months, Adequate bone marrow reserve (neutrophils $\geq 1.5 \times 10^3/\text{ml}$, platelets $\geq 100 \times 10^3/\text{ml}$, hemoglobin $> 9 \text{ gm/dl}$), hepatic profile (total bilirubin $\leq 1.5 \text{ unl}$ {upper normal level}, AST and ALT $\leq 2.5 \text{ unl}$, alkaline phosphatase $< 5 \text{ unl}$ except in presence of bone metastasis and in absence of any liver disorders, and renal functions (Creatinine $\leq 1 \text{ unl}$), and the calculated creatinine clearance should be $\geq 60 \text{ ml/min}$.)

All patients must have had previous treatment with anthracycline containing regimen either as adj. or neoadjuvant therapy and had no previous chemotherapy for MBC. Previous radiotherapy and hormonal therapy were permitted but hormonal therapy must be discontinued prior to enrollment.

Exclusion Criteria

Patients were excluded from the study if they had serious concomitant medical or psychiatric illness and prior or concurrent malignancy other than breast cancer. Patients with solitary brain and/or bone metastases and pregnant or lactating patients were considered ineligible for this study.

Pretreatment work up

Detailed history and clinical examination including:

Vital signs, systemic & locoregional examination, and performance status assessment. Body surface area for each patient will be calculated before initiation of treatment and every 2 cycles.

Laboratory studies

All patients performed the following Lab work prior to treatment initiation as well as prior to each chemotherapy cycle: Complete blood picture, liver function tests

(AST, ALT, T. bilirubin, alkaline phosphatase), renal function tests (S. creatinine and BUN, and creatinine clearance).

Radiological studies

All patients underwent full metastatic radiological work up for disease assessment and as a base line for response evaluation after therapy: chest x-ray (CT scan of the chest was done if x-ray revealed metastatic or suspicious lesions), CT scan of the abdomen and pelvis, Isotopic bone scan, and mammography and breast ultrasound. Other radiological investigations were done when clinically indicated.

Treatment strategy

All eligible patients fulfilling the inclusion criteria and after the pretreatment assessment received Gemcitabine 1250 mg/m^2 administered as a 30-minute intravenous infusion on days 1 and 8 plus cisplatin 75 mg/m^2 on day 1. The treatment cycle was repeated every 3 weeks. Cisplatin was administered intravenously during a 2-hour period of forced hydration with standard pre and post-treatment hydration. Infusion of 250 ml of 5% dextrose or normal saline containing 5-HT3 antagonist (8mg ondanestrone) and dexamethasone 8 mg over 30 minutes was administered before starting chemotherapy.

Outcome Assessment

Assessment of the response according to the WHO criteria¹⁴ was done every 2 cycles with repetition of clinical examination, lab work and routine imaging procedures that had been used to define the extent of the disease at presentation. The response to treatment reported here was the one measured at time of maximum radiological response.

All patients receiving ≥ 2 cycles and at least one re-evaluation are considered evaluable for response.

Duration of response

Complete response and partial response were calculated from the date of first assessment of response to the date of first progression or last follow up.

Time to progression (TTP)

was calculated from the date of inclusion in the study up to the date of last tumor assessment documenting first progression prior to start of further treatment.

Overall Survival

Survival was calculated from the date of study entry until the last follow up visit or death.

Toxicity

Toxicity was assessed using NCI criteria for common toxicities¹⁵ every cycle as well as on follow up.

Dose Modification for myelosuppression

Table 1: Shows dose modifications for gemcitabine and cisplatin according to Platelet and absolute neutrophil counts (% of Dose to be given)

HEMATOLOGIC TOXICITY				
Granulocytes				
Platelet Count (/mm ³)	>1500	1000-1500	500-1000	<500
>100,000	100	100	75	50
75,000–100,000	75	75	50	0*
50,000- 75,000	50	0	0	0
<50,000	0	0	0	0

*0 indicates treatment should be postponed a week until the counts return to a level at which drugs may be given. If no recovery after 2 weeks despite giving growth factors, the patient was taken off the study.

Dose modification for renal impairment

For cisplatin: In case of rise of serum creatinine > 1 normal value despite adequate rehydratin, creatinine clearance should be performed before each cycle and dose reduction should be considered. If creatinine clearance is 40-59(ml/min), 50% cisplatin dose at subsequent cycles, and if <40 (ml/min), the patient was taken off the study.

Dose modification for peripheral neuropathy

In case of grade II peripheral neuropathy, cisplatin will be reduced by 25% at subsequent cycles. In case of Grade III peripheral neuropathy, the patient was taken off the study.

Other toxic effects should be managed symptomatically if possible. In case of grade III toxicities chemotherapy would be held for a maximum of two weeks until resolution to grade ≤1, then given with 25% dose reduction. If grade IV toxicity occurs, except anemia and neutropenia, the patient was taken off the study. If a day 8 gemcitabine dose was held or missed, the cycle was continued per protocol with one dose not given. A patient who could not be administered day 8 of treatment for 6 weeks was taken off the study.

Treatment duration and follow up

A thorough examination was done for each patient in all visits, to be supported with radiological and laboratory investigations if needed.

Patients were treated till evidence of disease progression or unacceptable toxicity. Patients achieving CR continued two more cycles after remission with a minimum of 6 cycles and a maximum of 8 cycles. While patients achieving PR or SD continued 2 cycles after maximum response for a minimum of 6 cycles and a maximum of 8 cycles.

For responders who ended their maximum number of cycles, all clinical and radiological assessments were performed every 2-3 months for of all lesions till disease progression.

The patients who failed to respond to the above regimen or were removed from therapy because of progression, further antitumor therapy was allowed.

Statistical analysis

The data was collected, revised, coded and introduced to a PC. Data was statistically described in terms of range, mean, median, frequencies and relative frequencies. Regarding survival analysis, Kaplan Meier method¹⁶ was used. All statistical calculations were done using computer programs Microsoft Excel version 7 and statistical Package for Social Science program version 11 (SPSS package).

Results

This prospective phase II study included 40 female patients with MBC pretreated with anthracyclines as an adjuvant treatment who presented to Radiation Oncology and Nuclear medicine Department, Ain Shams University Hospitals, during the period from December 2006 to June 2009 with a median follow up period of 12 months ranging from 3 to 24 months. Patients' accrual was done from December 2006 to October 2007. Patients Characteristics are shown in table (2).

Table 2: Shows Patients' characteristic

Clinicopathological characteristics	Number of patients (percentages)	
Age (years):	< 40 40 - 49 ≥50	2 (5%) 17 (42.5%) 21 (52.5%)
Performance Status (ECOG):	0 1 2	15 (37.5%) 13 (32.5%) 12 (30%)
Menstrual Status:	Premenopausal Postmenopausal	11 (27.5%) 29 (72.5%)
Hormone Receptors:	ER+/ PR+ ER+/ PR- ER- /PR+ ER-/ PR-	12 (30%) 15 (37.5%) 4 (10%) 9 (22.5%)
Her2 status:	Positive Negative	18 (45%) 22 (55%)
Breast cancer phenotypes:	Her2+ Her2-/ HR+ Her2-/ HR-	18 (45%) 17 (42.5%) 5 (12.5%)
Disease free interval:	< 2 years ≥ 2 years	17 (42.5%) 23 (57.5%)
Number of metastatic sites:	1 2 ≥3	16 (40%) 20 (50%) 4 (10%)
Metastatic sites:	Liver Lung Others	8 (36.36%) 15 (68.18%) 9 (40.9%)

Regarding previous treatment, 72.5% of patients (29 patients) received FAC regimen as adj. chemotherapy and 27.5% (11 patients) received FEC regimen as adj. chemotherapy, while 77.5% of patients (31 patients) received adj. radiotherapy. Only 32.5% of patients (13 patients) received radiotherapy to metastatic disease before study entry.

About 77.5% of patients (31 patients) received adj. hormonal therapy and 12.5% of patients (5 patients) received hormonal therapy for metastatic disease prior to study entry.

Objective Tumor Response

Out of the forty patients recruited for the study, 38 patients were assessable for response because 2 patients were excluded from the evaluation of response. One patient suffered from sever toxicities (grade IV neutropenia and grade III vomiting) after the first cycle upon which she refused to tolerate further chemotherapy and the other patient suffered from unrelated sudden death (diabetes complication) after the first cycle. The overall objective tumor response (CR+PR) was 57.89%, while the clinical benefit ratio (CR+PR+SD) was 68.42%. Table 3

Table 3: Objective Tumor Response Rate

Response	Number of patients (Percentages)
Complete Response (CR)	2 (5.26%)
Partial Response (PR)	20 (52.63%)
Stationary Disease (SD)	4 (10.53%)
Progressive Disease (PD)	12 (31.58%)

The mean time to response was 2.681 months \pm 0.838 month as standard of deviation, the median time to response was 2 ms ranging from 2 to 4 ms with a 95% confidence interval(CI) of (2.34-2.96)ms and the mean cycle to response were 2.563 cycles \pm 0.791 cycle as standard of deviation, the median cycle to response were 2 cycles ranging from 2 to 4 cycles with a 95% CI of (2.29-2.97) cycles.

The mean duration of response was 9.934 ms \pm 4.309 ms as standard of deviation and the median duration of response was 9.5 ms ranging from 3 to 18 ms with a 95% CI of (8.07-11.83) ms.

The mean time to disease progression was 12.63 ms \pm 4.091 ms as standard of deviation and the median time to disease progression was 12.5 ms ranging from 5 to 20 ms with a 95% CI of (10.85-14.43) ms.

Table 4: Clinicopathological characteristics among responders

Clinicopathological characteristics	Number of patients (percentages)
Age (years):	
< 40	1 (4.54%)
40 - 49	11 (50%)
> 50	10 (45.46%)
Performance Status (ECOG):	
0	9 (40.91%)
1	9 (40.91%)
2	4 (18.18%)
Menstrual Status:	
Premenopausal	7 (31.82%)
Postmenopausal	15 (68.18%)
Hormone Receptors:	
ER+/ PR+	6 (27.27%)
ER+/ PR-	9 (40.91%)
ER- /PR+	2 (9.1%)
ER- /PR-	5 (22.72%)

Clinicopathological characteristics	Number of patients (percentages)
Her2 status:	
Positive	8 (36.37%)
Negative	14 (63.64%)
Breast cancer phenotypes:	
Her2+	8 (36.37%)
Her2-/ HR+	10 (45.45%)
Her2-/ HR-	4 (18.18%)
Disease free interval:	
< 2 years	6 (27.27%)
\geq 2 years	16 (72.73%)
Number of metastatic sites:	
1	12 (54.55%)
2	10 (45.45%)
\geq 3	0 (0%)
Others	9 (40.91%)

Survival

The estimated mean survival was 17.833 ms with a 95% CI of 15.459 to 20.207 ms. The estimated median survival was 22 ms with a 95% CI of 16.339 to 27.661 ms. (Figure 1)

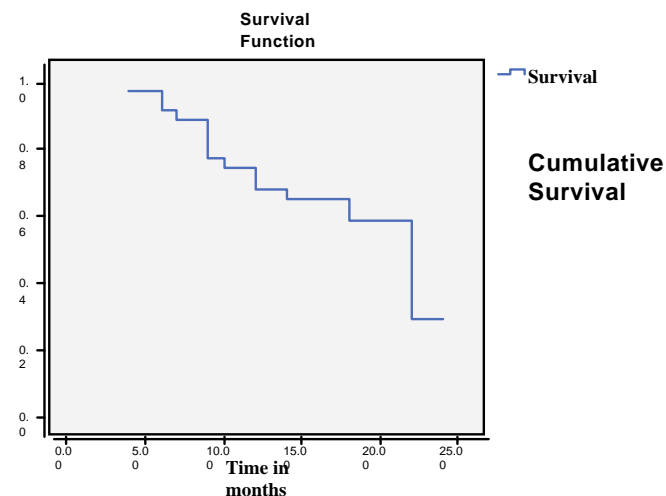


Fig 1: Survival function

From the survival curve, the survival function was estimated at 12 and 24 ms to be 68.52% and 31% respectively.

The estimated mean survival among responders and non-responders were 21.38 & 16.04 ms with a 95% CI of 19.57 to 23.18 & 13.39 to 18.69 ms respectively. The estimated median survival among responders and non-responders were 22 & 16 ms with a 95% CI of 16.84 to 27.15 & 12.89 to 19.10 ms respectively.

Comparative means for survival in relation to different clinicopathological characteristics (cancer phenotypes, performance status, age grouping, menopausal status and responders/non-responders) were done.

The triple negative cancer phenotype and better performance status among the study patients were associated with better survival; but this survival advantage did not reach statistical significance. However, significant survival advantage was observed among responders (P=0.006) Figure (2)

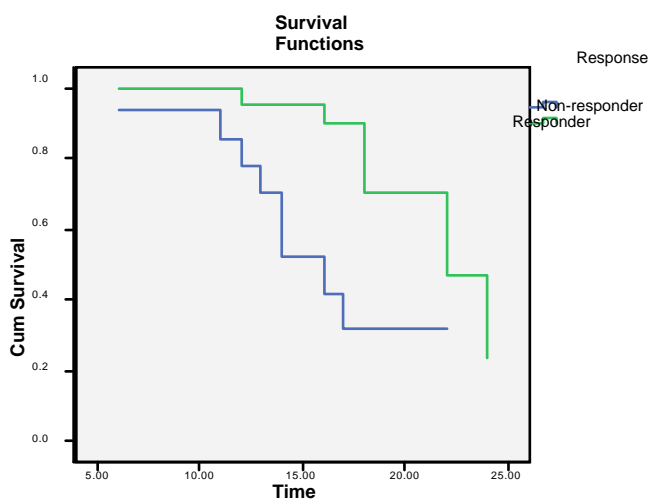


Fig 2: Survival functions in relation to responders/non-responders

Treatment Related Toxicity

The study regimen proved to be quite tolerable with the main hematological toxicities of this protocol were grade III anemia in 10% of patients, grade III/IV neutropenia in 20% of patients and grade III/IV thrombocytopenia in 17.5% of patients. Table (5)

Table 5: Hematological toxicity

Hematological Toxicity		Number of patients (Percentages)
Anemia	Grade I,II	21 (52.5%)
	Grade III,IV	4 (10%)
Neutropenia	Grade I,II	23 (57.5%)
	Grade III,IV	8 (20%)
Thrombocytopenia	Grade I,II	16 (40%)
	Grade III,IV	7 (17.5%)

There were no grade IV non hematological toxicity observed within the study and the only grade III non hematological toxicities recorded in the study were grade III nausea in 25% of patients, grade III vomiting in 17.5% of patients, grade III fatigue in 15% of patients and grade III renal toxicity in 2.5% of patients. Table(6).

Table 6: Most common treatment related non hematological toxicities

Toxicity		Number of patients (Percentages)
Nausea	Grade I,II	30 (75%)
	Grade III	10 (25%)
Vomiting	Grade I,II	23 (57.5%)
	Grade III	7 (17.5%)
Mucositis	Grade I,II	12 (30%)
Diarrhea	Grade I	3 (7.5%)
Alopecia	Grade I	5 (12.5%)
Hepatic toxicity	Grade I	2 (5%)
Renal toxicity	Grade I,II	7 (17.5%)
	Grade III	1 (2.5%)

Toxicity		Number of patients (Percentages)
Febrile neutropenia	Grade III	3 (7.5%)
Fatigue	Grade I,II	16 (40%)
	Grade III	6 (15%)
Neurosensory	Grade I,II	5 (12.5%)

Dose Intensity & Modifications

In total, 173 cycles were administered with a median of 5 cycles per patient (range 1–6). Of all the planned infusions, there were 15 dose reductions (8.67% of cycles) and 10 dose omissions (5.78%) for gemcitabine. Twelve patients (30%) had cycle delays.

Discussion

Sadly MBC is essentially incurable and all the treatment attempts will have more or less a palliative aim to it, which led to the absence of a standardized care for those patients and the subsequent emergence of QOL issues as the essence of MBC patients' care.¹⁷

Chemotherapy, targeted therapy and hormonal therapy, in addition to some surgical and radiotherapy maneuvers are the tools used either individually or combined; concomitantly or sequentially to control the metastatic disease according to a wide spectrum of clinical and pathological criteria.

The current phase II prospective single arm single institution trial was conducted to evaluate the usage of gemcitabine plus cisplatin as initial chemotherapy in women with MBC who had received prior anthracyclines in their adj. setting.

Out of the forty patients recruited to the current study, only 38 of them were evaluable for response. Around 5.2% of the patients achieved CR, 52.6% of the patients achieved PR, 10.5% of the patients showed SD and 31.6% of the patients had progressive disease. The overall objective tumor response was 57.9% while the clinical benefit ratio was 68.4%.

The median duration of response was 9.5 ms (95% CI, 8.07 to 11.83 ms) and the median time to disease progression was 12.5 ms (95% CI, 10.85 to 14.43 ms). The estimated median survival was 22 ms (95% CI, 16.34 to 27.66 ms). The 1 and 2 years survival probabilities were 68.52% and 31%, respectively.

These results were lower than the results achieved in a multicenter phase II study conducted by Fuentes and colleagues in which 46 patients with assessable MBC with previous exposure to anthracyclines received gemcitabine/cisplatin combination as first line chemotherapy.

Of the 42 evaluable patients, 17% of the patients achieved CR and 64% of the patients achieved PR while 19% of the patients had progressive disease. The overall tumor response was 81% (95% CI 69 to 93%).¹³

The Median TTP was 14.9 ms (95% CI 0 to 30.2 ms). The median duration of response was 24.2 ms (95% CI 11.2–37.3 ms). The estimated median survival was 27.9 ms (95% CI 23.1–32.7 ms), and the 1 and 2 year survival probabilities were 71.4% and 61.4%, respectively.¹³

These higher results could be attributed to the better clinico-pathological characteristics of the patients recruited in their study. The patients had an excellent Karnofsky performance status (83% of patients were 90% or above). In addition, only 35% of the patients had visceral metastasis and 40% of the patients had a single site of metastasis. Also only half of the patients received prior adj. anthracycline-based chemotherapy which made them more chemotherapy naive and probably more responsive. In comparison, to our current study where 70% of the patients had an ECOG performance status 0/1 and 80% of the patients had visceral metastasis.¹³

In another study where twenty two assessable patients for response were given gemcitabine/cisplatin as first line chemotherapy in MBC, 13.6% of the patients achieved CR, 40.9% of the patients achieved PR, 27.2% of the patients had evidence of SD and the remaining 18.2% of the patients had disease progression. The overall objective RR for the patients was 54.5% (95% CI, 38.3 to 76.7%). The overall objective RRs were quite comparable to our current study matched rates.¹² But their median duration of response being only 5 ms, median TTP of only 8 ms (95% CI, 7.2 to 10.8 ms) and median overall survival being 12.8 ms (95% CI 10.4 to 15.2 ms) were all lower than their matched results in our study.¹² The superiority of the current results as regard the duration of response and overall survival may be attributed to the higher number of recruited patients (40 patients compared to only 22 patients), also the better patients' clinicopathological characteristics.

In a multivariate analysis of our data a significant survival advantage was observed among responders, also a trend towards better survival was observed in patients with better ECOG performance status which is an established predictor of response.¹⁹ On the contrary, the menopausal status and the age of the patients recruited in our study did not have a significant effect on our study's survival. Triple negative breast cancer phenotype had a positive implication on survival of our study patients probably due to the platinum component of the protocol. This same observation led to initiation of an ongoing trial that is conducted to evaluate the role of gemcitabine/cisplatin combination as first line therapy in triple negative MBC patients.¹⁰

The results of the current study were superior to the results of a recent multi-institutional trial conducted by the US National Cancer Institute. In spite of being the largest published trial in advanced breast cancer that utilize the combination of cisplatin and gemcitabine in minimally and heavily treated MBC, the sophistication of the study design and its larger size, it failed to achieve the same responses achieved in the current study as a first line therapy.²⁰

In the NCI study, a total of 136 women were enrolled with similar patients' characteristics in both studies but Her2 status recognition was excluded from the NCI study. The overall RR was 26% for both the heavily pretreated subgroup (95% CI, 16% to 37%) and the minimally pretreated subgroup (95% CI, 16% to 38%) and the durations of response were 5.3 ms and 5.9 ms, respectively. The median OS rates were 10.8 ms (95% CI, 8.6 to 14.5 ms) in the heavily pretreated study and 13.1 ms (95% CI, 10.9 to 17.6 ms) in the minimally pretreated study. The median PFS rates were 3.8 ms (95% CI, 2.7 to 5.4ms) and 4.2 ms (95% CI, 3.6 to 6.0 ms) in the heavily and minimally pretreated studies, respectively. Interestingly, in a subgroup analysis that was performed for RR; HR- disease was associated with a higher RR that was not observed in the current study instead an increased survival ratio was observed in triple negative responders.²⁰

In a trial that recruited MBC patients to evaluate the role of gemcitabine/cisplatin as second line chemotherapy. The overall RR was 30% (95% CI, 12 to 53%). The median TTP was 30.6 weeks (95% CI, 12.6 to 44 weeks) and the median survival was 73.2 weeks (95% CI, 47.1 to 93.2 weeks) all are lower than our study's matched

data.²¹ All of these studies and more confirmed the superiority of the usage of the gemcitabine/cisplatin combination as first line therapy in comparison to its usage in minimally or heavily pretreated patients.

The North Central Cancer Treatment Group conducted a phase II study of weekly cisplatin and gemcitabine in a similar population of 58 patients with MBC and reported a RR of 29% and a median TTP of 6 months.²²

Another study of 38 patients with prior treatment with anthracyclines and taxanes reported a 40% overall RR with weekly cisplatin and gemcitabine as second-line therapy for MBC. The differences in RRs observed among these studies, including this study are most likely reflecting patient selection as well as differences in dosing and schedule.²³

Alternate dosing schedules included weekly lower doses of gemcitabine/cisplatin or a longer time of gemcitabine infusion were proposed by a series of studies. One trial used a weekly regimen of the combination and reported a similar RR to our study with RR of 50% in 30 heavily pretreated patients, but this regimen required modification because of cytopenias.²⁴

Among the forty patients assessable for toxicity, the major hematologic toxicities associated with the current study have been neutropenia and thrombocytopenia, and the major non-hematologic toxicities have been nausea/vomiting and nephrotoxicity.

Our current study was quite comparable to the two first line therapy trials as regard anemia. Ten percent of the patients developed Grade III anemia and none of the patients developed Grade IV anemia. Similarly, Grade III/IV anemia was seen in 9% of the patients in the study conducted by Fuentes. Grade III/IV anemia was seen in only 8% of the patients in Mohran's study.^{13,12}

Regarding neutropenia, the current study reported a lower incidence of neutropenia than the study conducted by Fuentes but a higher incidence than Mohran's study with 22.5% of patients developing Grade III/IV neutropenia. While in Fuentes study, 36% of patients developed grade III/IV neutropenia. Mohran's study reported a mere 12% of all his patients developing grade III/IV neutropenia. Neutropenic complications were comparable in all three studies.^{13, 12}

The higher percentage of neutropenia found in Fuentes et al, 2006 study could be explained by the higher number of cycles administered in his study.¹³

On contrary to neutropenia, Fuentes study reported less thrombocytopenia while Mohran's study reported more thrombocytopenia. Seventeen and half % of the current study patients developed Grade III/IV thrombocytopenia. Thrombocytopenia was the major hematologic toxicity observed in Mohran's study with grade III/IV thrombocytopenia in 32% of patients.^{13,12}

About 42.5% of patients developed Grade III nausea/vomiting. Both Fuentes et al., and Mohran's study recorded similar incidence of grade III nausea/ vomiting 33% and 32%, respectively.^{13, 12}

Twenty percent of the patients suffered from renal toxicity and only one of them developed grade III renal toxicity. While only 12% of Mohran's patients developed renal toxicity.¹²

The hematological and non hematological toxicities observed in trials using the gemcitabine/cisplatin combination as a second line or salvage therapy for MBC were higher than those observed in our trial.

The toxicities encountered in one trial were higher than our results with grade III/IV neutropenia in 36.4% of the patients compared to 22.5% and grade III/IV anemia in 25.6% compared to 10%.²¹

Toxicities in the NCI trial were mostly grade III/IV hematological toxicities which were much higher than our recorded toxicities with grades III/IV thrombocytopenia in 71% of patients compared to 17.5%, grade III/IV neutropenia in 66% of patients compared to 22.5% and grade III/IV anemia in 38% of patients compared to 10%. This high incidence of hematological toxicity is most likely the result of using the combination in heavily pretreated MBC patients with depleted bone marrow reserve. Prophylactic growth factors were a required element of the NCI protocol therapy for the heavily pretreated patients²⁰.

Conclusion

In conclusion, the study had found gemcitabine and cisplatin to be an effective and tolerable first-line treatment for MBC patients with impressive results compared to other first line therapies and superiority over the usage of the same combination as second line therapy.

However, because of the small size and patient selection of these studies, the findings should be validated in a larger randomized phase III studies which is also warranted to directly compare this regimen with widely used regimens to substantiate the best options for the treatment of patients in this setting.

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Prognostic factors of adult acute lymphoblastic leukemia and their impact on treatment outcome and long term survivals

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Abstract

Aim: To assess the prognostic factors of our adult ALL patients and their correlation to long term leukemia free (LFS) and overall (OS) survivals.

Patients and methods: Hundred and fifteen patients were included, they were stratified according to their prognostic factors into standard (SR), high (HR), and very high risk (VHR) groups.

Treatment plan:

Induction phase I: Vincristine, Doxorubicin, L-Asparaginase and prednisone with intrathecal MTX. Patients that attained CR were subjected to cranial irradiation and intrathecal MTX.

Phase II induction: Cyclophosphamide and Cytarabine.

Consolidation phase I: Vincristine, Doxorubicin and prednisone with Triple intrathecal.

Phase II consolidation: Cyclophosphamide, Cytarabine and Etoposide with triple intrathecal. **Maintenance therapy:** 6 mercaptopurine and methotrexate. For patients below 50 years with HR and VHR, one cycle of (HAM regimen) was added between induction and consolidation. VHR patients were referred to transplantation in CR1.

Results: The median age was 25 years. The study included 73 males and 42 females. Immunophenotypes were pro B (7%), CALL/Pre B (56.5%) and T phenotype (20.9%). The BCR-ABL and ALL1-AF4 fusion gene transcripts were positive in 15 and 4 of the precursor B cases respectively. Forty five patients were SR while 55 and 15 were HR and VHR respectively. CR was achieved in 76.5%. CR of the SR was 88.9% versus 70.9% and 60% for HR and VHR respectively. Median OS was 14 months. Survival at 60 months was 28.24 %, it was 34%, 21% and 20.7% for SR, HR and VHR respectively. There was significant survival difference between pro-B, Pre B /CALL and T phenotypes. Median time to progression was 16 months. At 60 months, 35.2% were still in remission. Time to progression was 44, 12 and 14 months for the SR, HR and VHR groups respectively, While it was 3, 17 and 16 months for Pro-B, pre-B/C-ALL, and T phenotypes respectively.

Conclusion: The CR, LFS and OS of the SR are satisfactory while those of the HR and VHR are still in need to be improved; whether we can achieve this by higher post remission chemotherapy, targeted therapy or stem cell transplantation remains to be investigated.

Introduction

ALL represents about 20% of adult leukemia. The median age in most registries varies between 25 and 35 years and 25% of patients are older than 15 years. ALL is diagnosed with an overall incidence of 1 to 1.5 per 100,000 population, but it has a bimodal age distribution: an early peak at around 4 to 5 years where the incidence may be as high as 4 to 5 per 100,000 population and a second gradual increase at around age 50 where it reaches up to 2 per 100,000 populations. (1, 2). For many years, adults with ALL have been treated with multi agent chemotherapy in the induction and post-remission settings. Most trials in adults were based loosely on regimens that were beneficial in children with high-risk disease, although at best, 40% of all adults with ALL achieved long-term disease-free survival, compared with 80% to 90% success rates in children with the same disease (3)

Despite improvements in the achievement of complete remission and progress in the supportive care of adults with acute lymphoblastic leukemia during the last decade, the majority of patients have eventually relapsed with overall survival of only 30-40%. However, the recent approach of adapting therapy according to biologic features appears to be resulting in significant progress for specific subsets of adults with acute lymphoblastic leukemia (4)

In the pre-imatinib era the prognosis for patients whose leukemic blast cells carry the BCR-ABL fusion created by t (9; 22) is poor with DFS rates of less than 10% with chemotherapy and 10% to 35% with allogeneic stem-cell transplantation. (5) The aim of this study was to improve the leukemia-free survival of our adult ALL Cases through more intensified post remission therapy and to weigh benefits versus risks of stratifying the patients to treatment protocol according to their prognostic criteria. We are also reporting, for the first time, in this study the 5 years LFS and OS figures of our cases.

Patients and Methods

Hundred and fifteen patients were included in this study between December 1999 and March 2004; patients were followed up for OS and LFS till March 2009. The diagnosis of ALL was performed according to standard clinical, radiological and laboratory workup. At presentation, patients were subjected to complete blood count, bone marrow examination and cytochemistry and immunophenotyping.

BCR / ABL as well as ALL1/AF4 fusion gene transcripts detection by RT – PCR were tested in the precursor B phenotype group. Exclusion criteria included mature B phenotype and age > 60 years. Before the start of treatment blood electrolytes and chemistries were done to test for organ functions and CSF examination for possible CNS involvement at presentation. The patients were then classified into standard, high and very high risk groups. The criteria for risk stratifications are summarized in table (1). The patient was considered high risk when at least one unfavorable criterion was present and very high risk when BCR / ABL fusion gene transcripts were detected by RT – PCR.

The treatment plan was risk adjusted, SR versus HR and VHR groups and was modified according to patient's age. Table (2), Fig (1)

Treatment regimen for the standard risk patients consisted of:-

Prephase for patients with TLC > 25 x 10⁹/L and/or organomegaly included: vincristine 2 mg Day 1 and prednisone 60 mg / m² P.O. Day 1 to Day 7.

Phase I Induction: Four drugs:

- Vincristine 1.4 mg/m² days 1, 8, 15, 22
- Daunorubicin 45 mg/m²I.V. days 1, 8, 15, 22
- L- Asparaginase: 5000 U/m²30 minute infusion days 15 to 28.
- Prednisone: 60 mg/m² P.O. day 1 through 28.
- Intrathecal methotrexate 15 mg on day 1.

Patients who attained CR after phase I induction were subjected to CNS prophylaxis including cranial irradiation (24 Gy) and intrathecal methotrexate 15 mg twice weekly for four injections. Phase II induction:

Started when neutrophils count is \geq 1500/L and platelet count is \geq 100 x 10⁹ / L and include:

- Cyclophosphamide 650 mg/m² I.V. short infusion on days 1, 14 and 28.
- Cytarabine 75 mg/m² short infusion four days a week for 4 weeks.

Phase I Consolidation :

- Vincristine: 2mg days 1, 8, 15, 22
- Adriamycin: 25 mg/m² days 1, 8, 15 and 22
- Prednisone: 60 mg/m² P.O. days 1 through 28 with gradual tapering.
- Triple intrathecal injection of: Methotrexate 15 mg, Cytarabine 40 mg and Dexamethazone 4 mg.

Phase II consolidation:

- Cyclophosphamide 650 mg / m² short infusion day 1.
- Cytarabine 75 mg/m² short infusion days 3, 4, 5, 6 and days 9, 10, 11, 12 then cytarabine 100 mg/m² short infusion days 25, 26, 27 and 30.
- Vepesid 100 mg/m² short infusion days 25, 26, 27 and 30.
- Triple intrathecal injection

Maintenance therapy:

Given for two years with:

- 6 mercaptopurine 60 mg / m² P.O daily
- Methotrexate 20 mg/m² I.V weekly.

Blood picture should be checked on a weekly basis.

- Maintenance triple intrathecal injection was given every two months till the end of maintenance therapy

HR and VHR patients above age of 50 years were treated as the standard risk group while those below age of 50 years, one cycle of high dose cytarabine and mitoxantrone (HAM regimen) was added between induction and consolidation.

HR and VHR cases below age of 50 years with available donor for transplantation were referred to allogeneic transplantation in CR1.

Imatinib methylate therapy was not given in this study as the study was planned before its establishment as an essential drug in the treatment of the VHR patients. Informed consents were signed by all cases.

Statistical Analysis

Statistical Package for social sciences (SPSS) version 12 was used for data analysis. Kaplan Meier was used for estimating survival and Log rank for comparing curves. P is significant at \leq 0.05.

Results

Between December 1999 and March 2004, 115 patients were included and followed up till March 2009. The median age was 25 years with a range of 16 to 60 years. Seventy three patients (63.5%) were males and 42(36.5%) were females. CNS disease at presentation was reported in 14 cases (12.2%).The patients' main clinical presentations are summarized in table (3).

The median TLC was 21 x 10⁹ /L (Range 0.5 to 423) with TLC \leq 30 x 10⁹ in 58.3% of cases. The median HB level was 7.4 gm /dl (Range 2.2 to 13.4) with HB level \leq 6 gm / dl in 38.3%. The median platelet count was 39.5 x 10³ /L (Range 10 to 330). Platelet count \leq 25 x 10⁹ /L was encountered in 34.3%. The median bone marrow blasts was 88 % (Range 32 to 99%). The immunophenotyping results were pro B in 8cases (7%), CALL/Pre B in 65cases (56.5%), and T phenotype was reported in 24cases (20.9%), the immunophenotype was not available in 18 cases (15.7%).The BCR-ABL fusion gene transcript was positive in 15 (20.5%) and ALL1-AF4 fusion genes transcripts was reported in 4 (5.47%) of the precursor B phenotype cases.

According to their prognostic factors, patients were stratified into SR group (n=45), HR group (n=55), and VHR group (n=15).The main reported toxicities of the induction phase are summarized in table (4)

Complete remission was achieved in 76.5% (n = 88). The CR rate was 88.9%, 70.9% and 60% for the SR, HR and VHR groups respectively (p=0.029).Only three cases, belonging to the very high risk group, were subjected to allogeneic transplantation as post-remission therapy.

The median survival for all patients was 14 months (95% CI, 9.2 to 18.8), survival at 36 and 60 months was 30.12 % and 28.24 % respectively. Survival curve for all patients is shown in fig (2). For the SR group, the median OS was 21months, with 3 and 5-years OS of 42.56% and 34% respectively. For the HR group, the median OS was 8 months with 3 and 5 -years OS of 21%. While the median OS of the VHR group was 7 months with 3 and 5-years OS rate of 20.7%.There was significant difference in OS between patients with SR, HR and VHR (P=0.017) (Fig 3). There was also a statistically significant difference in OS between patients with pro-B, pre-B/C ALL, and T phenotype (p=0.0019) (Fig 4) The median time to progression was 16 months (95% CI, 13.5 to 18.5) (Fig5).The LFS at 3 and 5 years was 40.6% and 35.2% respectively. Time to progression was 44, 12 and 14months for the SR, HR and VHR groups respectively (p=0.047) (Fig6). Time to progression of patients with Pro-B, pre-B/C-ALL, and T phenotype was 3, 17 and 16 months respectively (p=0.0007) (Fig 7)

Discussion

This study was conducted on 115 adult ALL patients. The median age was 25 years (range 16 to 60) in comparison to a median of 35(Range 15 to 65) in the

GMALL trial (6), and 33(Range 15 to 55) in the LALA-94 trial. (7). Age is probably the most important prognostic factor (3). Age of 35 years appears to be a clear prognostic cut off for adult ALL (8). OS continuously decreases with increasing age from 34-57% below 30 years to 15-17% above 50years (9, 10, 11&12). Although major improvement in survival was observed for patients less than 60 years, survival for patients aged 60 years or more remained unchanged at a level of around 10% (13). This group older than 60 years were treated with COAP regimen and were not included in this study. A male predominance was reported in our study with a male to female ratio of 1.74/1, which is nearly equal to the ratio of 1.82/1 reported in LALA-94(7) and documents the male predominance of ALL reported in our previous studies (14,15). Female gender is proposed to be one of the new clinical prognostic factors in recent analysis (16, 13). The median TLC at presentation in this study was $21 \times 10^9/L$ (0.5 – 423) which is nearly equal to a median of $22.4 \times 10^9/L$ (0.5-423 $\times 10^9/L$) reported by our previous study (15). Presenting TLC $\geq 30 \times 10^9/L$ for precursor-B cell and $\geq 100 \times 10^9/L$ for T cell ALL is of bad prognosis (8). In our study 41.7% of patients presented with a TLC $\geq 30 \times 10^9/L$ compared to 38% reported at the LALA-94 trial (7). In GMALL studies TLC $\geq 30 \times 10^9/L$ in precursor-B ALL (CALL/pre B) was considered the most deleterious prognostic factor with overall survival of 19-29% (6, 16), while in T –ALL the TLC was not a significant factor in multivariate analysis (6). High TLC may be associated with risk of complications during induction and higher risk of CNS relapse (16)

The immunophenotyping results in our study were pro B (7%), CALL/Pre B (56.5%), T phenotype was reported in 20.9%. these data are comparable to a precursor-B phenotype of 72% and a T phenotype of 26 % reported in the LALA-94 trial(7). Many groups confirmed the superior outcome of T cell compared to precursor-B cell phenotypes in adult ALL (6, 8&10). Pro B and/or t (4; 11) ALL is considered a poor prognostic subtype in many trials (16). In the GMALL, Pro B is considered an indication for SCT in CR1 (16) denoting its independent prognostic significance, a finding which is also documented in our study. CALL/Pre-B bears a large proportion of ph/BCR-ABL+ ALL and pre-B is associated with about 4% t (1; 19) (7, 16). According to the associated cytogenetic abnormalities Pre-B/CALL can be subdivided into high, very high or standard risk cases. The BCR-ABL fusion gene transcript was positive in 15 of our precursor B cases (20.5%) compared to 23% and 24 % Philadelphia positive in adult ALL reported by others(7,16). Ph+ ALL occurs in 20-30% of adult ALL with higher incidence in precursor B cases compared to 3-5% of children and it is generally associated with poor prognosis (5,13).The frequency of ALL1/AF4 fusion gene in the German series (17) is 6% which is comparable to 5.47% in ours. In our study 39% and 48% were allocated to the SR and HR groups, compared to 48% and 33% respectively in the GMALL studies (17)

Complete remission was achieved in 76.5% compared to 83% reported in GMALL trial (16) and 84% reported in LALA-94 trial (7). The CR rate of the large trials in adult ALL ranged between 74-93% with a mean of 83% (16). In the GMALL studies(16,17), the CR rate of the precursor-B lineage standard risk group was 88% compared to 87% in the T lineage standard risk which is comparable to the CR rate of 88.9% in our SR group. The CR rate of the HR and VHR groups in our study was 70.9 and 60 % respectively compared to 83 and 77% in the GMALL Trial (17). In our study the CR rate of the ph+ve group was 60% compared to 51% in the pre-imatinib era (18)

In our study the median OS for all patients was 14 months, survival at 36 and 60 months were 30.12 % and 28.24 % respectively. The median OS of the SR group was 21months, with 3 and 5years OS of 42.56% and 34% respectively. Median OS of the HR group was 8 months with 3 and 5-year OS of 21% while the median OS of the VHR group was 7 months with 3 and 5years OS rate of 20.7%. In the LALA-94 trial(7), the reported median OS was 23 months,

Patients with SR- ALL showed a Median OS of 37.8 months, with 3 and 5-years OS of 50% and 44% respectively. The median OS of the high risk patients was 29 months with 3 and 5-year OS of 46% and 38% respectively. While the Ph-positive group showed a median OS of 15.7 months with 3 and 5years OS rate of 28% and 24% respectively.

In our cohort, median time to progression was 16 months. At 3 and 5 years, 40.6% and 35.2% of the patients, respectively, were still in remission. There was significant difference in time to progression between patients classified as SR, HR and VHR (p=0.047). In LALA-94 trial the median LFS was 17.5 months with 3 and 5-years event free survival of 37 and 30% respectively and the estimated 3-year LFS rate was 35% and 43% for T- and (non-Ph-positive) B-lineage ALL respectively, (7).The LFS in large trials ranged between 27-48 % (at 3-10 years) with a weighted mean of 36 % (16). Only three VHR cases were subjected to transplantation. The lack of strict transplantation is related to several causes including lack of donor, impaired organ function, lack of financial coverage or refusal.

Conclusions and future direction

Our risk adapted protocol applied in this study was tolerable with accepted morbidity and mortality. Studying the prognostic criteria and stratification of the patients accordingly is mandatory. The integration of new risk factors as MRD detection and molecular genetic studies of the patients is evolving. The CR rate and LFS of the SR group are accepted and comparable to other studies with similar intensity, while those of the HR and VHR are still in need to be improved. Intensification of the post-remission chemotherapy, targeted therapy and strict referral to SCT are now considered in our ongoing protocol.

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Tables

Table 1: Criteria for risk stratification of adult ALL

	favorable	unfavorable
Age	≤ 35 years	> 35 years
WBC		
B lineage	< 30 x 10 ⁹ /L	> 30 x 10 ⁹ /L
T lineage	< 100 x 10 ⁹ /L	> 100 x 10 ⁹ /L
Immunophenotyping	Thymic T (T-lin. CD1a+ve or CD4/CD8 co-expression and sCD3-ve)	Pro B (B-lin. CD 10 -ve) Early T (T-lin. CD 1a -ve, sCD 3-ve). Mature T (T-lin. CD1a -ve , s CD3 + ve)
Molecular genetics		T (9,22) BCR/ABL T(4,11) ALL1/AF4
Treatment response	CR ≤ 4 weeks	CR > 4 weeks

Table 2: Summary of the treatment plan and modification according to age in adult ALL

Risk	Age < 50 years	50-60 years	>60 years
Standard risk	Standard risk protocol	Standard risk protocol	COAP regimen
High risk and very high risk	High risk protocol + allogeneic transplantation (in presence of HLA identical donor)	Treat as standard risk group and no transplantation	COAP regimen

Table 3: Baseline clinical presentations of 115 adult ALL cases

Fever and infection	22
Bleeding manifestations	27
Generalized lymphadenopathy	66
Mediastinal lymphadenopathy	6
Hepatosplenomegaly	65
CNS disease	14
Pleural effusion	6
Pulmonary infiltrates	6
Bone lesions	5
Impaired organ functions:	
-Hepatic	13
-Cardiac	1
-Renal	5

Table 4: Main reported toxicities of the induction phase in 115 adult ALL patients

Toxicity	Number
Fever and neutropenia	112 episodes
Mucositis	30
Diarrhea	12
Bleeding manifestations	9
Hepatic toxicity	21
Renal impairment	4
Diabetic keto-acidosis	2
Peripheral neuropathy	2

Figures

Fig 1: Risk-adapted treatment plan for adult ALL (other than mature B-ALL)

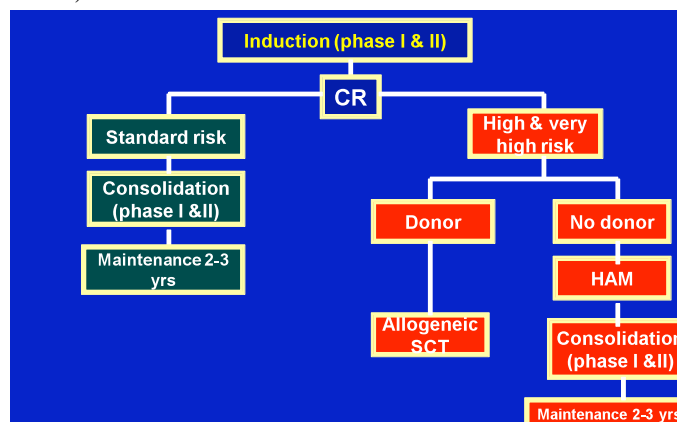


Fig 2: Overall survival of the whole group of ALL cases

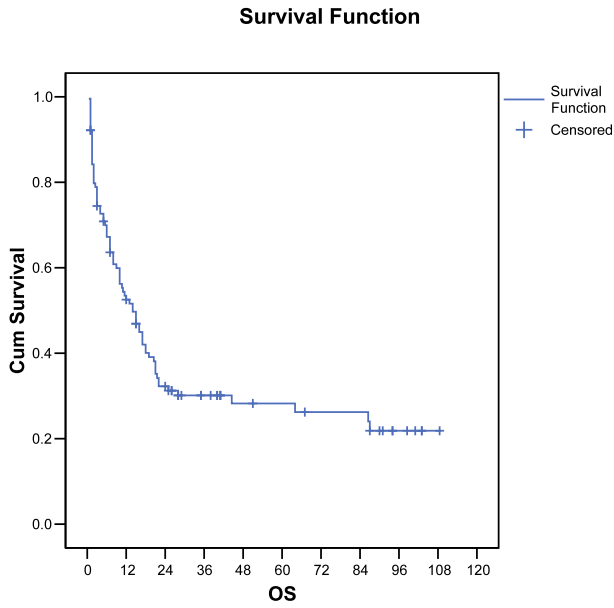


Fig 4: Overall survival according to immunophenotype

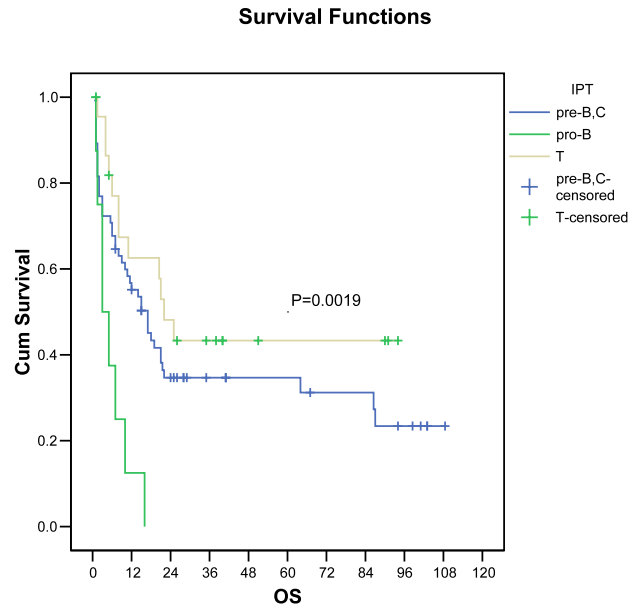


Fig 3: Overall survival according to risk groups

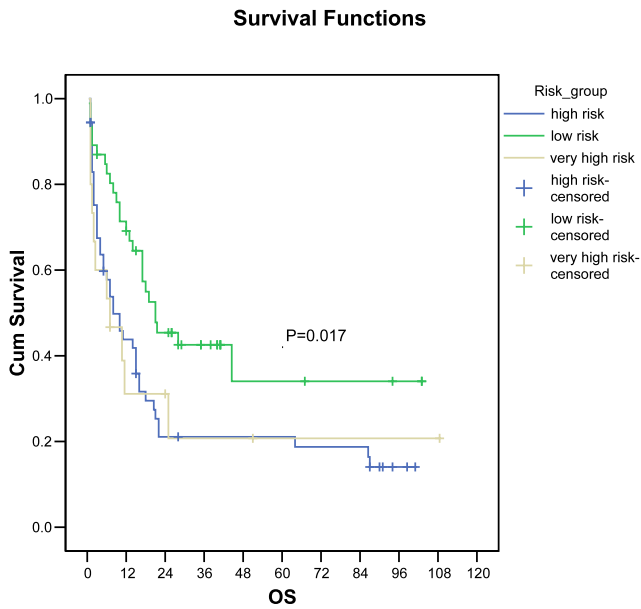


Fig 5: Disease free survival of the whole ALL group

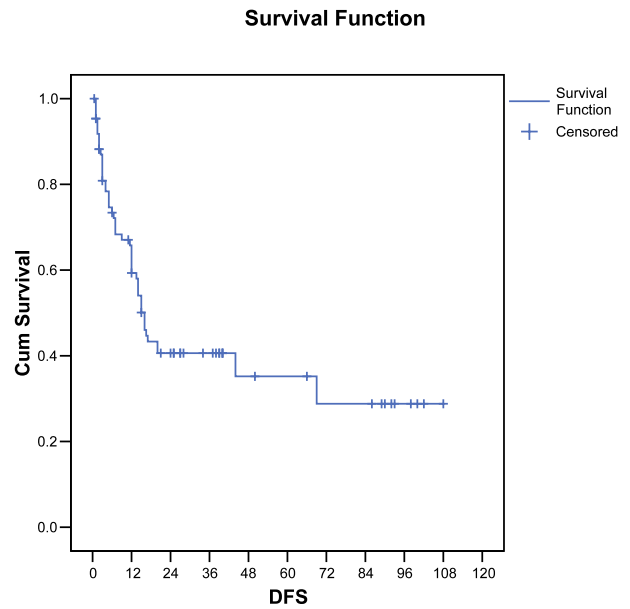


Fig 6: Disease free survival according to risk groups

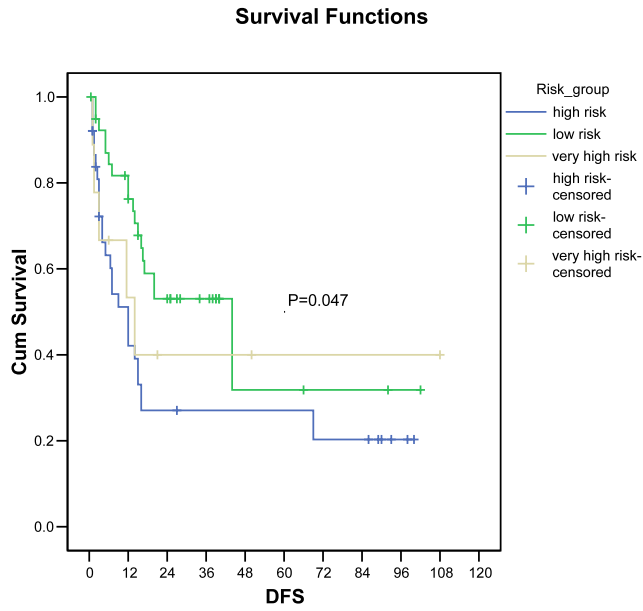
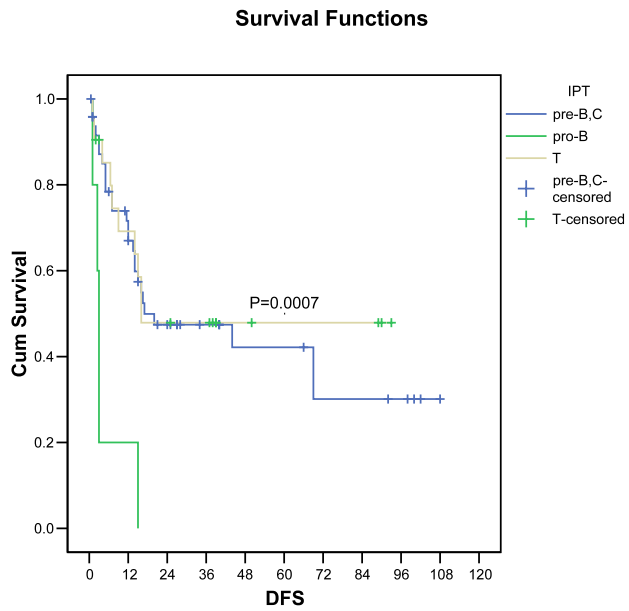


Fig 7: Disease free survival according to immunophenotype



Identification and clinical evaluation of the receptor for hyaluronic acid-mediated motility (RHAMM/CD 168) in AML patients

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Abstract

Purpose: this study was designated to detect RHAMM expression as an immunogenic antigen among Egyptian acute myeloid leukemia patients, regardless of their age, sex, concomitant cytogenetic abnormalities or FLT3-ITD status. RHAMM may be useful as an early diagnostic or prognostic marker and a potential target structure for cellular and antibody-based immunotherapies regardless its impact on the response to ttt. **Patients and Methods:** In the present study, RHAMM expression was tested in the peripheral blood samples of 40 AML patients as well as 20 healthy volunteers as a control group by RT-PCR. Patients were diagnosed according to the French-American-British cooperative group criteria. Their ages ranged between 12 and 71 years with a mean value of 37.4 ± 16.9 years. All patients were investigated at presentation prior to therapy. **Results:** The mRNA of RHAMM was detected in 24/40 (60%) of the patients while it was not detected in the control group. There were no statistically significant difference between RHAMM positive and negative patients as regard their clinical and laboratory data, although the highest remission rate was achieved in RHAMM positive patients while RHAMM negative patients had a higher rate of adverse treatment outcome (relapse and death during induction chemotherapy).

Conclusion: our findings are highly suggestive that the expression of RHAMM in newly diagnosed AML patients is a good prognostic marker as its expression is associated with favourable response to induction chemotherapy. Also being a tumour associated antigen, RHAMM represents a promising target for future immunotherapy.

Introduction

Acute myeloid leukaemia (AML) is a heterogeneous disease arising from clonal proliferation of neoplastic precursors in the bone marrow. A variety of prognostic factors have been identified that predict for outcome, most notably, the presence of defined cytogenetic abnormalities. Intensive combination chemotherapy treatment for acute leukaemia results in excellent remission rates as defined by <5% blasts in the bone marrow (1). Unfortunately, many patients relapse from a state of minimal residual disease (MRD), particularly patients in poor-risk categories. Even in the best prognostic categories, patients with AML have overall survival (OS) rates of (50–55%). Allogeneic stem cell transplantation is

associated with a lower risk of relapse (37–53%) compared with conventional chemotherapy, and results in improved disease-free survival rates in AML (2).

However, significant toxicity and transplant-related mortality limit the efficacy of this procedure and may abrogate the potential benefits of a lower relapse rate. The graft-vs-leukaemia effect mediated by donor cytotoxic T lymphocytes (CTLs) that potentially recognize and destroy the residual malignant cells is thought to be the mechanism by which disease control is achieved after allogeneic transplantation. This has led to the use of reduced-intensity conditioning allogeneic transplants and donor lymphocyte infusions (DLI) to utilize CTLs without the toxicity of conventional allogeneic transplantation. However, the use of allogeneic transplantation and DLI is still limited to a small number of suitable patients, and is complicated by the negative effects of alloreactive CTLs causing graft-vs-host disease due to their lack of specificity for the malignant clone (1).

Immunotherapy for leukaemia patients, aiming at the generation of anti-leukaemic T cell responses, could provide a new therapeutic approach to eliminate minimal residual disease (MRD) cells in acute myeloid leukaemia (AML). Leukaemic blasts harbour several ways to escape the immune system including deficient MHC class II expression, low levels of co-stimulatory molecules and suppressive cytokines (3). The development of cancer vaccines directed against myeloid leukaemias has been a research area of intense interest in the past decade. Both human studies in vitro and mouse models in vivo have demonstrated that leukaemia-associated antigens (LAAs), such as the fusion protein BCR-ABL, Wilms' tumour protein and proteinase 3, may serve as effective targets for cellular immunotherapy. Pilot clinical trials have been initiated in chronic and acute myeloid leukaemia and other haematological malignancies, which include vaccination of patients with synthetic peptides derived from these LAAs (4). Among these leukemia-associated antigens (LAAs) that induce a humoral immune response in AML patients the receptor for hyaluronic acid-mediated motility (RHAMM) (5,6).

RHAMM/CD168 is a receptor for hyaluronan, a glycoaminoglycan that plays a fundamental role in cell growth, differentiation and motility. RHAMM is a cell-surface receptor and belongs to the group of extracellular matrix molecules. The gene for RHAMM is localized on the human chromosome band 5q33.2. The expression pattern of RHAMM is highly tissue restricted. mRNA expression

of RHAMM was found in testis, placenta, thymus tissue and endothelium cells involved in angiogenesis. No expression of RHAMM was described on non-activated normal B cells in blood, spleen, or lymph nodes (5,7).

RHAMM is highly expressed in different tumor cell lines, also RHAMM mRNA and protein expression levels were detected in all leukemia cell lines. In non-haematological malignancies as melanomas, over-expression of RHAMM is essential for ras-mediated transformation and it is associated with the development of metastases. This demonstrates that RHAMM might be responsible for loss of control of the cell cycle and for the tendency of cancers to metastasize, which had been described earlier for other tumor entities as in melanomas (7).

RHAMM is an antigen eliciting both humoral and cellular immune responses in patients with AML, myelodysplastic syndrome and multiple myeloma (8). Giles *et al.* (9) demonstrated by immunocytologic staining that RHAMM/CD168 is expressed both in the cytoplasm and the surface of leukemic blasts, opening new strategies for potentially using RHAMM targeting antibody therapies.

The aim of this study is to detect RHAMM expression as an immunogenic antigen among Egyptian acute myeloid leukemia patients, regardless of their age, sex, concomitant cytogenetic abnormalities or FLT3-ITD status. RHAMM may be useful as an early diagnostic or prognostic marker and a potential target structure for cellular and antibody-based immunotherapies regardless its impact on the response to treatment.

Patients and Methods

The present study included 40 newly diagnosed AML patients. They were diagnosed at the departments of Medical Oncology and Clinical Pathology, Faculty of Medicine, Cairo University. Patients were diagnosed according to the French-American-British cooperative group criteria (10). Their ages ranged between 12 and 71 years with a mean value of 37.4 ± 16.9 years. All patients were investigated at presentation prior to therapy. Twenty age-sex matched healthy volunteers were included in this study as a control group. We didn't include more controls because RHAMM expression is tissue-restricted (testis, thymus and placenta) and is not normally detected in the peripheral blood mononuclear cells. All patients were subjected to the following:

- Full history taking, clinical examination with careful notation and assessment of clinical signs relevant to AML as: lymphadenopathy, hepatomegaly, splenomegaly, fever, fatigue, weight loss, jaundice, pallor, purpura, ecchymosis, easy bruising, recurrent infections and bone and joint pain.
- CT chest, abdomen and pelvis are done to assess lung, liver, spleen, lymph nodes and kidneys for possible pathological alterations.
- Cardiac examination including echo cardiography and ejection fraction to assess the cardiac condition of the patients that might be affected by anthracycline chemotherapy.
- Routine laboratory investigations as differential blood count (CBC), liver and kidney functions, serum uric acid, LDH and coagulation profile are done.
- In case of infection blood cultures and swabs are done.
- Bone marrow aspiration and cytochemical studies (including myeloperoxidase, non-specific esterase and dual esterase reactions).
- The immunophenotyping was done to establish the FAB subtyping.

- RHAMM gene expression in peripheral blood samples of patients and controls by conventional reverse transcriptase – polymerase chain reaction (RT-PCR).

Detection of RHAMM gene expression by RT-PCR as described by (7): Five ml of blood were withdrawn from every patient as well as the healthy volunteers in a sterile EDTA vacutainer. The mononuclear cells are separated and preserved at -20°C . Total cellular RNA was extracted from the mononuclear cells using the QIA amp RNA blood Mini kit (QIAGEN, Catalogue number. 52304), followed by c-DNA preparation using Revert Aid™ First strand cDNA synthesis kit (Fermentas, K1621). A volume of $5 \mu\text{l}$ cDNA was added to a final PCR reaction mixture of $25 \mu\text{l}$ containing $12.5 \mu\text{l}$ Master Mix (Fermentas K0171 which contains TaqDNA polymerase in reaction buffer, MgCl_2 and dNTPs), $1 \mu\text{l}$ of $10 \mu\text{M}$ of each of the forward and reverse RHAMM specific primers and $1 \mu\text{l}$ of $10 \mu\text{M}$ of each of the forward and reverse primers of β -actin. For standardization, expression of RHAMM was correlated with the expression of the house keeping gene β -actin. The primers for RHAMM: forward primer: $5' \text{- CAG GTC ACC CAA AGG AGT CTC G-3'}$, reverse primer: $5' \text{-CAA GCT CAT CCA GTG TTT GC-3'}$. For β -actin: forward primer: $5' \text{-GCA TCG TGA TGG ACT CCG-3'}$, reverse primer: $5' \text{-GCT GGA AGG TGG ACA GCG A-3'}$ (Fermentas™ – Germany). The following thermocycler program: initial denaturation at 95°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute. This was repeated for 36 cycles. The amplified products were separated on 2% agarose gel electrophoresis, stained with ethidium bromide. The electrophoretic pattern was visualized under UV light then photographed using a Polaroid camera with a red orange filter. The sample was considered positive when a clear sharp band was observed at the specific molecular weight; 661 bp for β -Actin and 565 bp for RHAMM.

Treatment and Assessment of the response to therapy:

Successful treatment of acute myeloid leukemia (AML) requires control of bone marrow, systemic disease and specific treatment of central nervous system (CNS) disease, if present. The cornerstone of this strategy includes systemically administered combination chemotherapy. Because only 5% of patients with AML develop CNS disease, prophylactic treatment is not indicated (11).

Treatment is divided into two phases: induction (to attain remission) and post-remission (to maintain remission). Maintenance therapy for AML was previously administered for several years but is not included in most current treatment clinical trials (12). Patients failing to respond to one or two cycles of such treatment are considered refractory. APL (FAB M3) induction chemotherapy included all-trans retinoic acid (ATRA) (13).

The induction chemotherapy regimen includes combination of mitoxantrone and Ara-C, in which Ara-C is given as $100 \text{ mg/m}^2 \text{ IV}$ by continuous infusion days 1-7 and mitoxantrone is given in a dose of 12 mg/m^2 intravenously daily for 3 days (7 and 3 regimen). Patients were admitted in the inpatient unit and they usually spend about one month in the hospital (14). Special consideration was given to induction therapy for acute promyelocytic leukemia (PML). Oral administration of all-trans-retinoic acid (ATRA) $45 \text{ mg/m}^2 \text{ /day PO}$ until CR induces remission in 70% to 90% of patients with M3 AML. ATRA induces terminal differentiation of the leukemic cells followed by restoration of nonclonal hematopoiesis¹⁸. The consolidation chemotherapy regimen includes high doses of Ara-C, in which Ara-C is given as 2 g/m^2 intravenous injections twice daily for 4 days. These were also given as inpatient treatments (15-17).

Adult acute myeloid leukemia (AML) in remission normalization of the

neutrophil count ($>1500 /\text{cm}^3$), platelet count ($>100.000/\text{cm}^3$), Cellular bone marrow with at least 20% cellularity, less than 5% blasts and no Auer rods, as well as absence of extramedullary infiltration. Resistance to induction is defined as more than 5% blasts in the bone marrow, lack of regeneration of normal haematopoiesis or evidence of extramedullary infiltration. Death during induction is defined as death during or after the first course of therapy with aplastic or hypoplastic marrow (18).

Statistical Analysis: Data were summarized and presented in the form of mean, range and standard deviation as descriptive statistics. Descriptive statistics and statistical comparison were performed using the statistical software program SPSS (version 15). Group comparison was done using either a 2-sample test or analysis of variance (ANOVA test). Correlation analysis was evaluated using the Pearson coefficient. Odds ratio and 95% confidence intervals (95% CI) were done for detection of the response to therapy. For all of the above mentioned statistical tests done, the threshold of significance is fixed at 5% level (p-value). Probability value (p-value) of more than 0.05 was considered non-significant, while p-value less than 0.05 indicated a significant result.

Results

The present study included 40 de novo AML patients, 22 males and 18 females. The main clinical and laboratory characteristics of AML patients were summarized in **Table (1)**. By conventional RT-PCR, mRNA of RHAMM gene was detected in 24/40 (60%) of the patients while it was not detected in the peripheral blood mononuclear cells of the control group. Comparison between RHAMM positive and negative patients regarding their clinical and laboratory findings is presented in **Table (2)**. There were no statistically significant difference between RHAMM positive and negative patients as regard their clinical and laboratory data. As regard the response to induction chemotherapy, the highest remission rate was achieved in RHAMM positive patients while RHAMM negative patients had a higher rate of adverse treatment outcome (relapse and death during induction) OR 2.455 and 95% CI 0.954 – 6.313 ($P = 0.035$) **Table (3)**.

As acute prolymphocytic leukemia (FAB-M3) has been considered as a separate disease entity among AML, and the response to treatment with all-trans retinoic acid (ATRA) had dramatically improved its clinical outcome, we omitted FAB-M3 cases (6 cases) from the patients group before evaluating their response to induction therapy.

RHAMM was expressed in 60% of our patients, and this high percentage should be taken in consideration for the importance of RHAMM peptide vaccine as an adjuvant therapy with chemotherapy or stem cell transplantation to eradicate residual leukemic cells and control minimal residual disease or augment graft versus leukemia effect after SCT.

Discussion

Treatment of patients with AML became more effective during the past decades, but a CR is often not durable and a high percentage of AML patients relapse. Therefore, complementary therapeutic approaches are under exploration for the prevention of relapse, and finding the reason for the unfavorable prognosis in AML patients. Specific immunotherapies for patients with acute myeloid leukemia (AML) using leukemia-associated antigens (LAA) as target structures

might be a therapeutic option to enhance the graft-vs.-leukemia effect observed after allogeneic stem cell transplantation or to prolong a complete remission (CR) achieved by chemotherapy (19,20).

Targeted immunotherapies require the identification and characterization of appropriate antigen structures. Initially, T-cell based cancer vaccines were designed for patients with solid tumors after the definition of suitable tumor-associated antigens. Several immunological and even clinical responses prompted researchers and clinicians to extend the spectrum of cancer vaccines towards hematologic malignancies such as acute myeloid leukemia (AML) (21). The identification of new immunogenic leukaemia-associated antigens (LAAs) is mandatory for the development of specific immunotherapies that selectively recognize and destroy leukaemia cells with the aim of reducing relapse rates without the need for allogeneic transplantation (1).

An ideal LAA that qualifies as a potential target for immunotherapies should be expressed preferentially in leukemic blasts, but neither on haematopoietic stem cells nor on normal tissues (22). Several leukemia-associated antigens (LAA) have been identified in patients with acute myeloid leukemia as BAGE, BCL-2, OFA-iLRP, FLT3-ITD, G250, hTERT, PRAME, proteinase 3, RHAMM, survivin, and WT-1 (23).

Excessive and detrimental vascularization occurs in neoplasia, promoting tumour growth and metastasis. Greater understanding of the mechanisms controlling the angiogenic process will provide mechanisms to inhibit neovascularization in tumours. The extracellular macromolecules, notably glycosaminoglycans (GAGs), are important mediators of angiogenesis. Hyaluronan (HA) is a large, non-sulphated GAG. Native high molecular weight HA (n-HA) is anti-angiogenic, whereas HA degradation products (o-HA; 3–10 disaccharides) stimulate endothelial cell (EC) proliferation, migration and tube formation following activation of specific HA receptors in particular, CD44 and Receptor for HA-Mediated Motility (RHAMM, CD168). Cell surface RHAMM and intracellular RHAMM are required for passage through G2/M phase of the cell cycle. Anti RHAMM antibodies blocked the migration of cells to the process of tissue injury and angiogenesis including EC, macrophages, smooth muscle cells and fibroblasts (23-26).

Greiner et al. (7) studied the mRNA expression of RHAMM in AML patients during their clinical course. After treatment of RHAMM positive patients with polychemotherapy, no mRNA expression of RHAMM was detectable. In all the peripheral blood mononuclear cells from the healthy volunteers, RHAMM was not expressed neither at the mRNA level nor the protein level.

In the present study, we focused on the influence of RHAMM expression on the response to induction chemotherapy of newly diagnosed AML patients, and also as a promising new target for future monovalent or polyvalent immunotherapeutic approaches. RHAMM expression was tested in the peripheral blood samples from 40 de novo AML patients as well as 15 healthy volunteers as a control group by conventional RT-PCR. All the control subjects were negative for RHAMM. This is in agreement with (5,7,19, 21,27).

Regarding the patient group, mRNA expression of RHAMM gene was detected in 24/40 (60%) of the patients. This is in accordance with (7,19,21) where RHAMM expression ranged between 60 to 70% of their patients. *Greiner et al.* (7) reported that RHAMM expression at the protein level was higher than the mRNA level (70% vs 60%). However, the percentage of RHAMM

positive patients was higher in the study of *Greiner et al.* (5) as RHAMM was expressed in more than 80% of their AML patients by RT-PCR and Western blot techniques. This may be attributed to the small patient group enrolled in this study (17 patients).

Comparing RHAMM positive with RHAMM negative patients in the present study, no statistically significant difference was found between the two groups regarding the age, the presenting symptoms or the incidence of hepatomegaly, splenomegaly or lymphadenopathy. RHAMM expression was more prominent among male patients however, the difference between the two groups did not reach a statistically significant level ($p=0.072$). This may be attributed to the relatively small number of patients under study. This is in agreement with (4,19) who reported that RHAMM expression did not correlate to age, sex or clinical presentation of the patients. On the other hand, *Greiner et al.* (27) reported that there was a significant association between ages more than 60 years and 'no TAAs' expression, as older age is known to be associated with adverse outcome.

No significant differences were detected as for the expression of RHAMM in the FAB subtypes M1 - M5. This is in accordance with (5,19,21) where no significant difference was noted in their studies as regards FAB subtypes M0 - M5. Although RHAMM expression was more prominent among FAB M1 patients yet it was statistically insignificant.

Correlation of RHAMM expression with the response to induction chemotherapy revealed that RHAMM positive patients reached a statistically significant higher complete remission rate ($p=0.035$). This is in accordance with (7,19,27). *Greiner et al.* (5) reported that most of the patients enrolled in their study showed complete remission of their disease following induction chemotherapy. Also, *Greiner et al.* (21) reported that RHAMM expression is a good prognostic marker, associated with favourable treatment outcome.

The expression of tumour associated antigens might play a critical role in the control of minimal residual disease, and therefore might be associated with the clinical outcome in AML. RHAMM, PRAME and G250 can induce strong antileukemic immune responses, possibly controlling MRD control. High expression of at least one of the three antigens, RHAMM, PRAME or G250 provided the strongest favourable prognostic effect. Thus, these tumour associated antigens represent interesting targets for polyvalent immunotherapeutic approaches in AML (21).

The lack of RHAMM mRNA expression in CD34+ haematopoietic stem cells or in normal tissues (except for testis, placenta and thymus) renders it a potent immunologic target for the immune system to fight a residual tumour load following chemotherapy in AML (27). Immunotherapy targeting RHAMM might be an option to enhance such a specific antileukemia (GVL) effect after chemotherapy or allogeneic transplantation without aggravating graft-vs-host disease (GVHD). Moreover, the therapies might be a useful tool to prolong the duration of complete remission reached by induction chemotherapy. RHAMM is currently used in peptide vaccination trials for patients with haematological malignancies (RHAMM-R3 peptide vaccination) (8,28).

In conclusion, our findings are highly suggestive that the expression of RHAMM in newly diagnosed AML patients is a good prognostic marker as its expression is associated with favourable response to induction chemotherapy. Also being a tumour associated antigen, RHAMM represents a promising target for future vaccination trials to further augment immune responses relevant in AML therapy.

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Tables

Table 1: The main characteristics of AML patients included in the present study

	AML patient (Number = 40)	
Age (years)	12 – 71 (37.4 ± 16.9) years	
Sex (M/F)	Males= 22 / 40 (55%) Females= 18 / 40 (45%)	
Lymphadenopathy (number -%)	Absent	24/40 (60%)
	Present	16/40 (40%)
Hepatomegaly (number -%)	Absent	30/40 (75%)
	Present	10/40 (25%)
Splenomegaly (number -%)	Absent	18/40 (45%)
	Present	22/40 (55%)
Bleeding tendency (number -%)	Absent	14/40 (35%)
	Present	26/40 (65%)
Fever (number -%)	Absent	13/40 (32.5%)
	Present	27/40 (67.5%)
Haemoglobin level (gm/dl) (range, mean ± SD)	5 – 11 (7.53 ± 1.28)	
Total leucocytic count (X10 ³ /cmm) (range, mean ± SD)	4 – 509 (58.35 ± 107.89)	
Platelet Count (X10 ³ /cmm) (range, mean ± SD)	8 – 91 (37 ± 27.41)	
Peripheral blood blasts (%) (Range, mean ± SD)	10 – 100% (53.5 ± 31.27)	
Bone marrow blasts (%) (Range, mean ± SD)	35 – 100% (76.35 ± 24.35)	
FAB classification (Number - %)	M1= 14 / 40 (35%)	
	M2= 8 / 40 (20%)	
	M3= 6 / 40 (15%)	
	M4= 8 / 40 (20%)	
	M5= 4 / 40 (10%)	
RHAMM expression by (RT-PCR)	Negative = 16 / 40 (40%)	
	Positive = 24 / 40 (60%)	
Response to induction chemotherapy	Complete remission = 15 / 40 (37.5%)	
	Relapsed = 3 / 40 (7.5%)	
	Death = 22 / 40 (55%)	

Table 2: Comparison between RHAMM positive and negative patients by RT-PCR regarding their clinical and laboratory data

		RHAMM Positive (n = 24)	RHAMM Negative (n = 16)	P value	Significance	
Clinical Data	Sex:					
	Male	16 / 24 (66.7%)	6 / 16 (37.5%)	0.072	NS	
	Female	8 / 24 (33.3%)	10 / 16 (62.5%)			
	Age (years) (range, mean ± SD)	12 – 63 (34 ± 16.65)	17 – 71 (42.43 ± 16.55)	0.13	NS	
	Splenomegaly	-	12 / 24 (50%)	6 / 16 (37.5%)	0.43	NS
		+	12 / 24 (50%)	10 / 16 (62.5%)		
	Hepatomegaly	-	18 / 24 (75%)	12 / 16 (75%)	1	NS
		+	6 / 24 (25%)	4 / 16 (25%)		
Lymph-adenopathy	-	16 / 24 (66.7%)	8 / 16 (50%)	0.29	NS	
	+	8 / 24 (33.3%)	8 / 16 (50%)			
Fever	-	9 / 24 (37.5%)	4 / 16 (25%)	0.43	NS	
	+	15 / 24 (62.5%)	12 / 16 (75%)			
Bleeding tendency	-	8 / 24 (33.3%)	6 / 16 (37.5%)	0.78	NS	
	+	16 / 24 (66.6%)	10 / 16 (62.5%)			
Laboratory Data (Mean ± SD)	Hb (g/dl)	7.7 ± 1.48	7.25 ± 0.88	0.26	NS	
	WBC (x103/cm3)	73.7 ± 135.81	35.26 ± 32.17	0.269	NS	
	Platelet (x103/cm3)	40.8 ± 30.9	31.25 ± 20.53	0.279	NS	
	PB blast (%)	53.5 ± 30.9	53.6 ± 32.75	0.99	NS	
	BM blasts (%)	75.3 ± 24.3	77.8 ± 25.04	0.74	NS	
FAB Classification	M1	8 / 24 (33.3%)	6 / 16 (37.5%)	0.78	NS	
	M2	4 / 24 (16.7%)	4 / 16 (25%)	0.52		
	M3	4 / 24 (16.7%)	2 / 16 (12.5%)	0.71		
	M4	6 / 24 (25%)	2 / 16 (12.5%)	0.32		
	M5	2 / 24 (8.3%)	2 / 16 (12.5%)	0.67		

- : Absent, + : Present

NS= Statistically not significant, S= Statistically significant.

Table 3: RHAMM gene expression in relation to the response to induction chemotherapy

Response to induction chemotherapy of non-M3 cases	RHAMM + (n=20)	RHAMM- (n=14)	P-value	significance
CR	10/20 (50%)	2/14 (14.2%)	0.043	significant
Failed induction	2/20 (10%)	1/14 (7.1%)		
Death during induction	8/20 (40%)	11/14 (78.5%)		

RHAMM + M3 cases = 4, all achieved CR.

RHAMM – M3 cases= 2, 1 died from DIC and 1 CR.

The highest remission rate was achieved in RHAMM + non-M3 (PML) cases.

Figures

Fig 1: RHAMM gene expression by RT-PC

1 2 3 4 5 6 7 8



-Upper panel: Amplification with RHAMM specific primers showing 565 bp PCR product. Lane 4,5,7 show +ve expression, Lane 2,3,6,8 show negative expression.

- Lower panel: the same RNA as in lanes given above amplified with β -actin specific primers (661 bp) serves as an internal control for the quality of RNA.

- Lane 1; 100- 1500 bp ladder size marker.

Primitive neuroectodermal tumour of the orbit: A case report and literature review

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Abstract

Primitive neuroectodermal tumours are rare. The orbital localization is exceptional. Only 13 cases were described in the literature; which only three are adults. We report a case of a 59-year-old man, who was admitted for exophthalmia, NMR revealed a tumour in the left orbit. Pathological examination and Immunohistochemical studies showed a Primitive neuroectodermal tumour. The patient was treated with chemotherapy followed by radiation therapy. After 22 months of follow-up, the patient remained free of disease

Introduction

Primitive neuroectodermal tumour (PNET) is a term used to describe a category of highly Malignant small round cell tumours of neuro-ectodermal origin with variable cell differentiation [1, 2]; primitive neuroectodermal tumours are rare, they represent less than 1 % of all the sarcomas.

The majority of PNET occurs in the central nervous system (SNC).

PNET recognized outside of the CNS are diagnosed as peripheral PNET. The orbital localization is exceptional only 13 cases have been reported previously; we present the clinical, radiological and histopathological features of an orbital mass in a 59 year-old man.

Observation

A 59-year-old man presented with a 10-month history of exophthalmia.

Nuclear magnetic resonance NMR revealed a heterogeneous hyper intense signal on a T2-weighted image in the left orbit.

Pathological examination showed a sheet of small cells with irregular nuclei. Immunohistochemical studies demonstrated positive immunoreactivity for neurone-specific enolase and synaptophysin, On the basis of these findings, a diagnosis of primitive neuroectodermal tumour of the orbit was made. The patient was treated with three courses of chemotherapy consisting of, ifosfamide, etoposid doxorubicin and cysplatin, the response was estimated at 50 % by NMR. Then he was treated with 60 Gray of external beam radiation therapy (2Gray per fraction and 30 fractions). After 22 months of follow-up, the patient remained free of disease and a repeat NMR was normalized, with no sign of residual tumour.

Discussion

Primitive neuroectodermal tumours are very uncommon.

The orbital localization is exceptional; only 13 cases have been reported in the literature [1, 3].

The value of this reported case is related to his age, since all reported cases in the literature are pediatrics, except 3 patients, and all the three are man. (Table 1).

Table 1: table including all reported cases in the literature starting from 1986

Authors name	Case age	Case sex	Year of publication	Reference
S Shuangshoti and al	52-year-old	man	1986	Br J Ophthalmol. 1986 July; 70(7): 543-548.
Wilson WB and al	7-year-old	girl	1988	Cancer. 1988 Dec 15;62(12):2595-601
Singh AD and al	10-year-old	girl	1994	Arch Ophthalmol. 1994 Feb;112(2):217-21.
Bansal RK and al	8-month-old	boy	1995	Indian J Ophthalmol 1995;43:29-31.
Kiratli and al	28-year-old	man	1999	Ophthalmology. 1999 Jan; 106(1):98-102.
Alyahya GA and al	5-year-old	girl	2000	Graefes Arch Clin Exp Ophthalmol. 2000 Sep;238(9):801-6.

Authors name	Case age	Case sex	Year of publication	Reference
Sen S and al			2002	J Pediatr Ophthalmol Strabismus. 2002 Jul-Aug;39(4):242-4.
Lezrek M and al	13-year-old	Boy	2005	J Fr Ophtalmol 2005; 28: e8.
Romero R and al	6-year-old	Boy	2006	Arch Soc Esp Oftalmol. 2006 Oct; 81(10):599-602.
Znati K and al	23 year-old	man	2006	Revue Française des Laboratoires, Volume 2006, 380, March 2006, 41-43.
Tamer C and al	10-year-old	boy	2007	Can J Ophthalmol. 2007 Feb;42(1):138-40
Romero IL and al	10-month-old	girl	2008	Arq Bras Oftalmol. 2008 Nov-Dec;71(6):871-3.
Kim UK and al	2 year old	girl	2009	Indian ophtal J; 10; 2009; 200-202.

Conclusion

Primitive neuroectodermal tumour of the orbit is extremely rare, especially in adults. the differential diagnosis of PNET of the orbit includes the other small blue round cell tumours. Treatment is based on chemotherapy associated to radiation therapy or surgery. Prognosis is very poor.

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Figures

A computed tomography scan showed a left orbital mass



Radiotherapy by 2 Fields, anterior and left side Field.



The diagnosis is always based on histological analysis and immunohistochemical staining with or without cytogenetically study.

Histologically, PNETs are highly cellular and demonstrate a monotonous pattern of small round cells with hyper chromatic nuclei, and high nuclear–cytoplasm ratio with varying degrees of neuronal differentiation. This progressive process begins with neuron specific enolase (NSE) expressivity, followed by Homer–Wright rosette formation, phenotypic ganglion cell differentiation, and finally by neurofilament protein expression [4, 5].

The differential diagnosis of PNET of the orbit includes the other small blue round cell tumours: lymphoma neuroblastoma, Ewing's sarcoma, rhabdomyosarcoma, small cell osteogenic sarcoma, and mesenchymal chondrosarcoma [1.6].

There is no established treatment protocol for patients with orbital PNET ,these tumours progress rapidly and often have already metastasized at the time of diagnosis [7].Because of the close relationship with Ewing Sarcoma, supplementary chemotherapy, radiation therapy or both is currently recommended [8,9.10].

These tumours prognosis is poor; their evolution is marked by local recurrences and metastasis. Survival ranged from 1 month to 5 years. For 6 cases of 13 cases of orbital PNET report in the literature, the survival was between 6 and 45 months.

Nasopharyngeal adenoid Cystic carcinoma: About tow cases and review of the literature

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Abstract

Nasopharyngeal adenoid Cystic carcinoma is a rare tumour; few cases were reported in the literature. It is characterized by slow growth rate, high tendency to local recurrence and metastatic spread. The corner stone of its therapeutic management is surgery when is possible, unfortunately because of difficulty of access to the nasopharynx; surgery is more often limited; so radiotherapy seems to remain the ideal treatment. The chemotherapy is indicated in the metastasis situations.

We report two cases aged respectively: 50, and 46 years old. The first patient presented with diffuse pulmonary metastasis; the treatment combined chemotherapy, based on cisplatin and adriamycin drugs, and palliative radiation therapy at the dose of 30 Gy. The patient died 6 months after diagnosis. The second patient, presented with locally advanced disease, was treated by external beam radiotherapy at the dose of 70 Gy. 36 months after the end of the treatment, she presented a local recurrence treated by re-irradiation of the nasopharynx at the dose of 60 Gy. The patient is still alive without disease 12 months after the treatment completed.

Introduction

Previously termed “cylindroma” by Billoth in 1856, the adenoid cystic carcinoma (ACC) of the nasopharynx is a rare disease with a slow growing, but locally aggressive and thus prone to recurrence. Another important characteristic is its tendency to infiltrate neural structures and to spread perineurally [10]. We report two cases about this rare localization.

Case 1: A 50 years old female admitted to our Hospital with headache and epistaxis for 5 months duration. Otorhinolaryngological examination showed a nasopharyngeal tumour extended to the oropharynx without palpable lymph nodes. Computed tomography scan of the head and neck showed a big tumor on the right side wall of the nsopharynx. This tumour was extended to the right pterygoid fossa and towards the sphenoidal sinus, and filling the ethmoidal cells, without endocranial extension (fig1). The nasopharyngeal biopsy revealed invasive adenoid cystic carcinoma (solid type). The radiological assessment of the disease found diffuse pulmonary metastasis (fig 2). The patient was managed with combined chemotherapy based on cisplatin 100mg/m² and doxorubicin

50mg/m² repeated every 3 weeks. However, the assessment of the disease after 2 cycles of chemotherapy showed tumour progression in the nasopharynx and in the lung. Then, the treatment consisted of palliative nasopharyngeal radiotherapy (30Gy) in 10 fractions, and best supportive care. The patient died six months later with severe haemoptysis.

Case 2: A 46 years old female without any medical history, admitted to our institution with nasal obstruction, epistaxis and right hypoacusia during 8 months. Otorhinolaryngological examination showed tumour of the right superior-side wall of the nasopharynx without palpable adenopathy. The CT scan of the head and neck revealed a tumour at the nasopharyngeal superior and right walls (fig 3). Biopsy of the nasopharynx found adenoid cystic carcinoma infiltration. Radiological assessment of tumour with chest radiography and abdominal ultrasound imaging showed no abnormalities. The patient was treated successfully with radiotherapy (70 Gy) in 35 fractions. However, she presented a local recurrence 36 months later. Then, the management consisted of reirradiation at the dose of 60 Gy in 30 fractions. The patient is still alive without evident disease 12 months after the treatment completed.

Discussion

Adenoid cystic carcinoma (ACC) is a malignant tumour of the exocrine glands. It most commonly arises in the salivary glands, even if localizations have been described in prostate, lacrymal glands, uterine cervix, breast and bronchial mucosa [7]. Nasopharyngeal localization is uncommon, accounting for 0.5% to 4% of all nasopharyngeal carcinomas and for 2.4% to 3.7% of all head and neck ACC [1].

In the nasopharynx, ACC occurs most frequently in the 5th decade of life, without sex predilection [5]. The symptoms most commonly found are epistaxis, progressive nasal stenosis, dysfunction of the Eustachian tube and, in relation to the invasion of the skull base, disorders of ocular motility, diplopia, facial pain, dysfunction of IX, X, XI and XII pairs of cranial nerves and, more rarely, Horner's syndrome [8].

Imaging of ACC is based on computed tomography (CT) scan, particularly helpful in detecting bony erosions of the skull base, and on Magnetic Resonance Imaging (MRI) with gadolinium, effective in demonstrating possible involvement of infra-temporal fossa, cavernous sinus, and perineural or

perivascular infiltration [8].

Histologically, three subtypes have been described by Perzin and al [5] in 1978, reflecting various degrees of progression of cellular differentiation, as well as aggressiveness of biologic behaviour. The tubular subtype, which is the most differentiated form and the 2nd most frequent (30%), presents a recurrence rate more than 50% and an overall 9-year survival. The cribriform subtype, which is less differentiated than the tubular and the most common (50%) for most authors, presents a 90% recurrence and an 8-year overall survival. The cribriform and tubular subtypes manifest a tendency for local infiltration. The less differentiated and thus the most malignant and aggressive is the solid subtype, accounting for 10% of the cases. It often gives distant metastases (70% overall) and mainly invades lung, brain and bones and has by far the poorest prognosis [5].

As Adenoid Cystic Carcinoma has low sensitivity to Radiotherapy [4,6], surgical treatment is the main treatment policy for patients with limited NACC mainly in stage I, II, and III [2,9]. Wen et al. [10] showed the 5-year OS of the surgical treatment group was 50.0%, which was significantly higher than that of the radiotherapy group (38.5%). Mendenhall et al. [16] studied the treatment results of 101 patients with head and neck adenoid cystic carcinoma, and he found the 10-year rates of local control of the radiotherapy group and the surgery plus radiotherapy group were 43% and 91%, respectively; moreover, the 10-year absolute survival rates were 42% and 55%, respectively. Therefore, the optimal treatment policy for patients with NACC may be surgery plus radiotherapy. Patients with incomplete resection or with advanced tumors had bad prognosis. However, recent study from Schramm and Imola [9] had demonstrated that combined extensive surgical resection and radiation therapy might achieve survival outcomes in nasopharyngeal ACC comparable with treatment results reported about Adenoid Cystic Carcinoma of other sites in head and neck region. Chemotherapy has a limited role in the ACC of the nasopharynx and its use is still a matter of discussion. Cisplatin, 5-Fluorouracil, Doxorubicin and others are used in combination with radiotherapy, with reports of some success and remissions [5].

Conclusion

Nasopharyngeal adenoid Cystic carcinoma is rare. Its biological behaviour is characterized by slow growth, high tendency to local recurrence and metastatic spread. Its histological features are particularly important for prognostic prediction: solid pattern has the worst outcome. The corner stone of its therapeutic management is surgery. Radiotherapy improves the local control of the disease and chemotherapy is helpful in treating the metastatic disease.

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Figures

Figure 1: CT scan showing a tumor at the right side wall of the nasopharynx

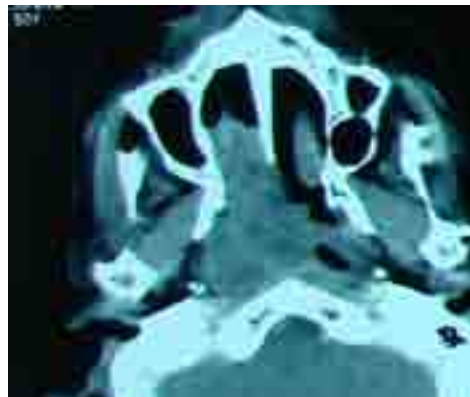


Figure 2: Chest radiography showing diffuse metastasis in both lungs

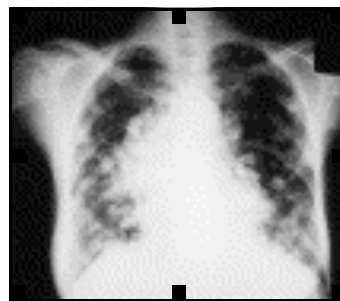
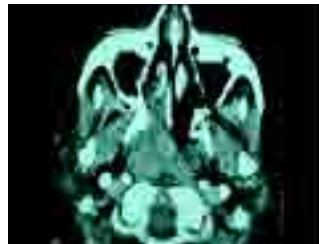


Figure 3: Axial CT showing a tumor of the right side wall of the nasopharynx



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LEBANESE SOCIETY OF MEDICAL ONCOLOGY

the 3 G's debates:
Case based discussions in
**Gastrointestinal, Genitourinary and
Gynecologic Oncology**

LSMO National Forum
December 3-5, 2010
Beirut - Lebanon



Lebanese Society of Medical Oncology
الجمعية اللبنانية لأطباء الأورام الخبيث



fom

Francophones
d'Oncologie
Médicale

Formation pratique et actualisée de
haut niveau ciblée sur l'innovation
dans les traitements médicaux des
différents types de cancer, destinée
aux oncologues francophones.



Les informations relatives au Congrès seront
régulièrement mises à jour sur le site Internet:

13-15 JANVIER
2011
LILLE GRAND PALAIS

www.fom-k.net

fom-k@clq-group.com

A large rectangular area with rounded corners, outlined in blue, containing 25 horizontal dotted lines for writing notes.



FOM 2010

les points-clés

Le premier congrès Francophone d'Oncologie Médicale (FOM) a eu lieu à Lille du 14 au 16 Janvier 2010...

Premières journées internationales de formation médicale des spécialistes francophones du cancer, c'est samedi soir 16 Janvier 2010 que ce sont achevées les FOM. Quelques **300 oncologues médicaux** et spécialistes d'organes étaient réunis à Lille Grand Palais, **français mais aussi médecins francophones venus d'autres pays** : Belgique, Suisse, Canada, Liban, Roumanie, Hongrie, République Tchèque, et Tunisie étaient représentés.

En trois jours de travail, cette première édition a été un succès notamment au plan scientifique avec **75 présentations regroupées en 10 thématiques principales** : cancer du sein, du poumon, cancers digestifs, gynécologiques, urologiques, des voies aéro-digestives supérieures (VADS), mélanomes et sarcomes, tumeurs cérébrales, mais aussi nanotechnologies, et nouveaux médicaments contre le cancer.

Dans un programme dense, **présidé par le Professeur Jacques Bonnetterre de Lille**, l'innovation médicale et son intégration dans la pratique des spécialistes ont été abordées dans leurs multiples facettes.

Les **mots-clés** de ces messages scientifiques resteront : **thérapeutique ciblée, individualisation du traitement, marqueurs moléculaires, et bien sûr optimisation des résultats pour le patient.**

Quelques exemples parmi les sujets traités :

- Dans le cancer du poumon, la relation entre les mutations des récepteurs à l'EGF et la réponse au traitement par les thérapeutiques ciblées
- Pour les sarcomes, les possibilités d'individualisation du traitement en fonction des sous-types de tumeurs maintenant mieux diagnostiqués

- Dans le cancer du sein, la place de l'IRM dans le bilan initial de la patiente, et l'étude des caractéristiques des tumeurs dites « triple négatives » dont le pronostic est particulièrement mauvais

- Dans certains cancers ORL, la relation avec une infection par le Papilloma virus humain, ou HPV, comme cela est déjà connu pour le cancer du col utérin.

- Dans les tumeurs cérébrales, les modalités d'utilisation en pratique clinique des modifications géniques comme la délétion des chromosomes 1p et 19q.

- Dans le cancer rénal, l'arrivée de l'évérolimus, un 5^{ème} traitement innovant là où rien n'existait il y a seulement quelques années

- Dans les hépatocarcinomes, la place de nouvelles thérapeutiques ciblées comme le sorafénib a été présentée, ainsi que le rôle possible du trastuzumab dans les tumeurs gastriques

- Dans les tumeurs gynécologiques, une place prometteuse des inhibiteurs de PARP dans la stratégie de prise en charge des tumeurs récidivantes ;

- Les nanotechnologies offrent elles aussi de très intéressantes perspectives, actuellement en évaluation chez les patients, d'une chirurgie plus sélective grâce à une meilleure détection des limites de la tumeur.

- Enfin, du côté des **nouvelles molécules et futurs traitements**, les équipes de Lille, Bruxelles, Lyon et Villejuif ont présenté les nouvelles méthodes de développement dites phases 0, les nouvelles études de phase 1 qui permettent un gain de temps et de bénéfice pour le patient.

De même, l'efficacité des traitements par les anti-angiogéniques peut être à présent évaluée par des techniques d'imagerie de contraste, donc optimisée dans la pratique.

Ces rencontres entre spécialistes du cancer ont été l'occasion d'échanges, de nouveaux projets et collaborations, qui seront relayés toute l'année sur le site Internet des FOM : www.fom-k.net, site où seront aussi disponibles les communications scientifiques présentées au congrès.



Les FOM sont parrainées par le Ministère des Affaires Etrangères / Secrétariat d'Etat chargé de la Coopération et de la Francophonie, par l'Institut National du Cancer (INCa), et par l'Université de Lille Nord de France, mais ont aussi reçu une **triple accréditation** en tant que formation médicale continue : du Conseil National de la FMC pour la France, de la Société Européenne d'Oncologie Médicale (ESMO) et de l'ACOE / UEMS pour l'Europe.

L'organisation des FOM a été **soutenue par l'industrie pharmaceutique, et 28 sponsors** ont permis la création de ce nouveau congrès, en particulier les Laboratoires AstraZeneca, Bayer Schering Pharma, et Roche. Fortes de leur succès et de l'intérêt de cette nouvelle formule d'échanges entre spécialistes du cancer, les FOM auront désormais lieu **chaque année à Lille**, et vous donnent **rendez-vous en Janvier 2011**.

PROGRAMME 2011

3 journées en 10 thématiques : 9 sessions scientifiques + 1 conférence invitée + 10 sessions sponsorisées

Horaire	Jeudi 13 Janvier 2011	Vendredi 14 Janvier 2011	Samedi 15 janvier 2011
8h	Accueil	Accueil	Accueil
8h30	Session ONCOLOGIE THORACIQUE	Conférence invitée "Cancer et Environnement"	Pt déjeuner-débats
9h30		Session ONCOLOGIE DIGESTIVE	Session SENOLOGIE
10h30			
11h	Session CANCERS DES VADS	ONCOLOGIE DIGESTIVE (suite)	SENOLOGIE (suite)
12h30	Déjeuner-débats et cas cliniques	Déjeuner-débats et cas cliniques	Déjeuner-débats et cas cliniques
14h	Session NOUVELLES MOLECULES	Session NEURO-ONCOLOGIE	Session SOINS DE SUPPORT
15h30			Fin du congrès
16h	Session IMMUNO-ONCOLOGIE	Session ONCOLOGIE GYNECOLOGIQUE	
17h30	Cocktail FOM	Débats et cas cliniques	
18h30			

JANUARY	Cervical Cancer Awareness Month
FEBRUARY	Screening and Early Detection Awareness Month
MARCH	Colorectal Cancer Awareness Month
APRIL	Cancer Fatigue Awareness Month
MAY	Melanoma and Skin Cancer Awareness Month
JUNE	National Cancer Survivors Day
JULY	Sarcoma Awareness Month
AUGUST	Pain Medicine and Palliative Care
SEPTEMBER	Gynecologic Cancer Awareness Month Prostate Cancer Awareness Month Leukemia and Lymphoma Awareness Month
OCTOBER	Breast Cancer Awareness Month
NOVEMBER	Lung Cancer Awareness Month Smoking Cessation
DECEMBER	5 A Day Awareness Month

OBJECTIVES & SCOPE OF THE PAJO ◀

The Pan Arab Journal of Oncology (PAJO) is the official Journal of the Arab Medical Association Against Cancer (AMAAC). It is a quarterly publication targeting health professionals interested in the oncology field. It is a multidisciplinary peer-reviewed journal that publishes articles addressing medical oncology, malignant hematology, surgery, radiotherapy, pediatric oncology, geriatric oncology, basic research and the comprehensive management of patients with malignant diseases in addition to international oncology activities, congresses & news.

The journal will be addressed, as a first step, mainly to the professionals in the hematology & oncology field in the Middle East region and North Africa. The goal is to share local & regional research activities news and to be updated with international activities. We hope, with your support, to achieve our following objectives:

1. Promote and encourage research activities in the Arab World.
2. Disseminate & analyze epidemiological local, regional and international data.
3. Update health professionals with the most recent advances, news & developments in the field of oncology.
4. Improve the level of scientific publications arising from the Arab World.
5. Keep health professionals connected and exposed to the activities of different Arab cancer societies.
6. Share with our immigrant compatriots their activities & feedback in this field.
7. Involve all health professionals interested in the field of Oncology within the multidisciplinary scope of the Journal.
8. Encourage post graduates students to submit their research work.

INSTRUCTIONS FOR AUTHORS ◀

1. Manuscript Categories

1.1. Clinical trials

The Editor-in-Chief and an Associate Editor generally review Reports from clinical trials. Selected manuscripts are also reviewed by at least two external peer reviewers. Comments offered by reviewers are returned to the author(s) for consideration.

Manuscript acceptance is based on many factors, including the importance of the research to the field of oncology & the quality of the study. Authors should focus on accuracy, clarity, and brevity in their presentation, and should avoid lengthy introductions, repetition of data from tables and figures in the text, and unfocused discussions. Extended patient demographic data should be included in a table, not listed within the text.

Reports from Clinical trials are limited to 3,000 words of body text, excluding the abstract, references, figures, and tables. They are limited to six total figures and tables. All abstracts are strictly limited to 250 words. Titles are to be descriptive, but succinct.

Results of clinical studies should be supported by a clear description of the study design, conduct, and analysis methods used to obtain the results.

Reports of phase II & III studies should include from the protocol a clear definition of the primary end point, the hypothesized value of the primary end point that justified the planned sample size, and a discussion of possible weaknesses, such as comparison to historical controls.

Phase I studies will be well received if they have interesting clinical responses, unusual toxicity that pointed to mechanism of action of the agents, and important or novel correlative laboratory studies associated with the trials.

1.2. Review Articles

All reviews must be clinically oriented, ie, at least half the review must describe studies that detail human impact, marker effect on prognosis, or clinical trials.

Review Articles should be prepared in accordance with the Journal's Manuscript Preparation Guidelines, and will be reviewed in the same manner as Reports from Clinical Trials. Reviews are limited to 4,500 words of body text, excluding the abstract, references, figures, and tables. The editors also suggest a limit of 150 references.

1.3. Editorials / Comments / Controversies

The Editor-in-Chief may solicit an Editorial to accompany an accepted manuscript. Authors who wish to submit unsolicited Comments and Controversies should contact the Editor-in-Chief, before submission to determine the appropriateness of the topic for publication in the Journal.

Editorials should be no more than four to five pages in length.

1.4. Articles on Health Economics

Articles about health economics (cost of disease, cost-effectiveness of drugs, etc) are highly encouraged.

1.5. Case Reports / Correspondence / Special Articles

Correspondence (letters to the Editor) may be in response to a published article, or a short, free-standing piece expressing an opinion, describing a unique case, or reporting an observation that would not qualify as an Original Report. If the Correspondence is in response to a published article, the Editor-in-Chief may choose to invite the article's authors to write a Correspondence reply. Correspondence should be no longer than three pages in length. Special Articles present reports, news from international, regional societies as well as news from our compatriots.

2. Manuscript submission procedure

All manuscripts should be submitted in word and PDF format directly to the Editor-in-Chief by e-mail at the following e-mail: editorinchief.pajo@yahoo.com.

The manuscript should adhere to the journal requirements. Upon manuscript submission, corresponding authors must provide unique e-mail addresses for all contributing authors. Receipt of manuscripts will be acknowledged via e-mail. Upon completion of editorial review, the corresponding author will receive notification of the Editor's decision, along with the reviewers' comments, as appropriate, via e-mail.

3. Disclosures of Potential Conflicts of interest

In compliance with standards established and implemented by ASCO's Conflict of Interest Policy (J Clin Oncol 24:519–521, 2006), it is the PAJO's intent, as previously referred, to ensure balance, independence, objectivity, and scientific rigor in all of its editorial policies related to the Journal through the disclosure of financial interests, among other measures. All contributors to the Journal are required to disclose financial and other relationships with entities that have investment, licensing, or other commercial interests in the subject matter under consideration in their article. These disclosures should include, but are not limited to, relationships with pharmaceutical and biotechnology companies, device manufacturers, or other corporations whose products or services are related to the subject matter of the submission.

Disclosures of financial interests or relationships involving the authors must be addressed on the Author Disclosure Declaration form. The corresponding author may complete the form on behalf of other authors, or authors may complete their own forms and forward them to the corresponding author. This information will be sent to the Editorial Board. Statements regarding financial support of the research must be made on the manuscript title page, and disclosed on the form. This form is available upon request from the Editorial Office. All disclosures will appear in print at the end of all published articles.

The Journal requires all Editors and reviewers to make similar disclosures. Reviewers are asked to make disclosures when accepting a review.

4. Manuscript Preparation Guidelines

Title Page

The first page of the manuscript must contain the following information: (1) title of the report, as succinct as possible; (2) author list of no more than 20 names (first name, last name); (3) names of the authors' institutions and an indication of each author's affiliation; (4) acknowledgments of research support; (5) name, address, telephone and fax numbers, and e-mail address of the corresponding author; (6) running head of no more than 80 characters (including spaces); (7) list of where and when the study has been presented in part elsewhere, if applicable; and (8) disclaimers, if any.

Abstract

Abstracts are limited to 250 words and must appear after the title page. Abstracts must be formatted according to the following headings: (1) Purpose, (2) Patients and methods (or materials and methods, similar heading), (3) Results, and (4) Conclusion. Authors may use design instead of Patients and methods in abstracts of Review Articles. Comments and Controversies, Editorials and Correspondence do not require abstracts.

Text

The body of the manuscript should be written as concisely as possible and must not exceed the manuscript category word limits described herein. All pages of a submission should be numbered and double-spaced. Helvetica and Arial at 12pt size are the recommended fonts for all text (see Figures section for acceptable fonts for figures). The Journal adheres to the style guidelines set forth by the International Committee of Medical Journal Editors.

References

References must be listed and numbered after the body text in the order in which they are cited in the text. They should be double-spaced and should appear under the heading "REFERENCES." Abbreviations of medical periodicals should conform to those used in the latest edition of Index Medicus and on MEDLINE. The «List of Journals Indexed in Index Medicus» includes the latest abbreviations. Inclusive page numbers must be cited in the reference. When a reference is for an abstract or supplement, it must be identified as such in parentheses at the end of the reference. Abstract and supplement numbers should be provided, if applicable. When a reference is a personal communication, unpublished data, a manuscript in preparation, or a manuscript submitted but not in press, it should be included in parentheses in the body of the text, and not cited in the reference list. Published manuscripts and manuscripts that have been accepted and are pending publication should be cited in the reference list.

Reference Style

° Journal article with one, two, or three authors

1. Dolan ME, Pegg AE: O6-Benzylguanine and its role in chemotherapy. Clin Cancer Res 8:837-847, 1997

° Journal article with more than three authors

2. Knox S, Hoppe RT, Maloney D, et al: Treatment of cutaneous T-cell lymphoma with chimeric anti-CD4 monoclonal antibody. Blood 87:893-899, 1996

° Journal article in press (manuscript has been accepted for publication)

3. Scadden DT, Schenkein DP, Bernstein Z, et al: Combined immunotoxin and chemotherapy for AIDS-related non-Hodgkin's lymphoma. Cancer (in press)

° Supplement

4. Brusamolino E, Orlandi E, Morra E, et al: Analysis of long-term

results and prognostic factors among 138 patients with advanced Hodgkin's disease treated with the alternating MOPP/ABVD chemotherapy. *Ann Oncol* 5:S53-S57, 1994 (suppl 2)

° Book with a single author

5. Woodruff R: *Symptom Control in Advanced Cancer*. Victoria, Australia, Asperula Pty Ltd, 1997, pp 65-69

° Book with multiple authors

6. Iverson C, Flanagan A, Fontanarosa PB, et al: *American Medical Association Manual of Style* (ed 9). Baltimore, MD, Williams & Wilkins, 1998

° Chapter in a multiauthored book with editors

7. Seykora JT, Elder DE: Common acquired nevi and dysplastic nevi as precursor lesions and risk markers of melanoma, in Kirkwood JM (ed): *Molecular Diagnosis and Treatment of Melanoma*. New York, NY, Marcel Dekker, 1998, pp 55-86

° Abstract

8. Bardia A, Wang AH, Hartmann LC, et al: Physical activity and risk of postmenopausal breast cancer defined by hormone receptor status and histology: A large prospective cohort study with 18 years of follow up. *J Clin Oncol* 24:49s, 2006 (suppl; abstr 1002)

9. Kaplan EH, Jones CM, Berger MS: A phase II, open-label, multicenter study of GW572016 in patients with trastuzumab refractory metastatic breast cancer. *Proc Am Soc Clin Oncol* 22:245, 2003 (abstr 981)

° Conference/meeting presentation

10. Dupont E, Riviere M, Latreille J, et al: Neovastat: An inhibitor of angiogenesis with anti-cancer activity. Presented at the American Association of Cancer Research Special Conference on Angiogenesis and Cancer, Orlando, FL, January 24-28, 1998

° Internet resource

11. Health Care Financing Administration: Bureau of data management and strategy from the 100% MEDPAR inpatient hospital fiscal year 1994: All inpatients by diagnosis related groups, 6/95 update. <http://www.hcfa.gov/a1194drg.txt>

° Digital Object Identifier (DOI)

12. Small EJ, Smith MR, Seaman JJ, et al: Combined analysis of two multicenter, randomized, placebo-controlled studies of pamidronate disodium for the palliation of bone pain in men with metastatic prostate cancer. *J Clin Oncol* 10.1200/JCO.2003.05.147

° Government Announcement/Publication

13. Miller BA, Ries CAG, Hankey BF, et al (eds): *Cancer Statistics Review: 1973-1989*. Bethesda, MD, National Cancer Institute, NIH publication No. 92-2789, 1992

° ASCO Educational Book

14. Benson AB 3rd: Present and future role of prognostic and predictive markers for patients with colorectal cancer. *Am Soc Clin Oncol Ed Book* 187-190, 2006

Figures

Figures must be cited in the order they appear in the text using Arabic numerals. Figures should be submitted in a separate document. Figure legends are required for all article types. Figure legends must not exceed 55 words per figure and should be written below the figure.

Images may be embedded in word or Power Point files.

Tables

Tables must be cited in the order in which they appear in the text using Arabic numerals. The table's legend may include any pertinent notes and must include definitions of all abbreviations and acronyms that have been used in the table. Tables submitted with multiple parts will be renumbered. Tables should be submitted in a separate document. Legends must not exceed 55 words per table and should be written above the figure.

Appendices/Acknowledgments

Appendices and acknowledgments will appear in the print version of the article.

Language: Appropriate use of the English language is encouraged for publication in the Journal.

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