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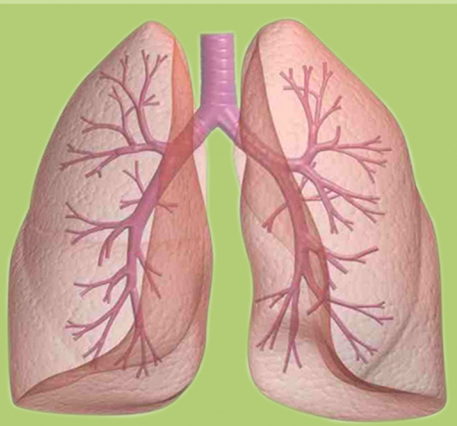

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Case Report

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Hemothorax Caused by Primary Pulmonary Angiosarcoma

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ABSTRACT

A previously healthy 57-year-old woman was admitted to the Emergency Department with hemothorax. She had continuously blood loss during the next 24 hours accordingly she went to the OR in order to achieve hemostasis.Intraoperative findings were multiple lesions in the lung parenchyma and diffuse bleeding from multiple small abnormal nodules on the surface of the visceral pleural and parietal pleura.Biopsies prompted the diagnosis of angiosarcoma and with the primary tumour burden in the lung parenchyma the pathologist diagnosed a primary pulmonary angiosarcoma.The disease was widespread at the time of diagnosis accordingly curative intended treatment was not an option and because of her general poor condition she died one day after discharge.

KEYWORDS: Primary pulmonary angiosarcoma; Intrathoracic bleeding; Hemothorax.

INTRODUCTION

Sarcoma in general is very rare tumour contributing with only 0.7% of all malignant tumours while angiosarcoma is even rarer contributing with only 1% all sarcomas. The presence of an angiosarcoma in the lung is usually a consequence of metastasis from another primary site but in very few cases it has been described with a primary origin in the lung.

CASE REPORT

A previously healthy 57-year-old woman was admitted to the Emergency Department with shortness of breath and in general poor conditions. The oxygen saturation was 91%, blood pressure 113/68 mmHg, and heart rate 119 beats per minute. Blood samples revealed hemoglobin (Hb) at 4.2 mmol/l (reference 7.0-10.0), white blood cell count 24.3 x 10^9/l (3.0-10.0) and C-reactive protein 128 mg/l (<10). A chest X-ray at the primary survey demonstrated pleural effusion on the right side (Figure 1).

A 24 Charrière chest tube was inserted, with immediate drainage of about 1200 ml coagulated blood. Anemia and the general poor clinical condition demanded blood transfusions. The patient had continuously blood loss (over 3000 ml) and decreasing hemoglobin (Hb 3.4 mmol/l) during the next 24 hours leading to a thoracotomy.

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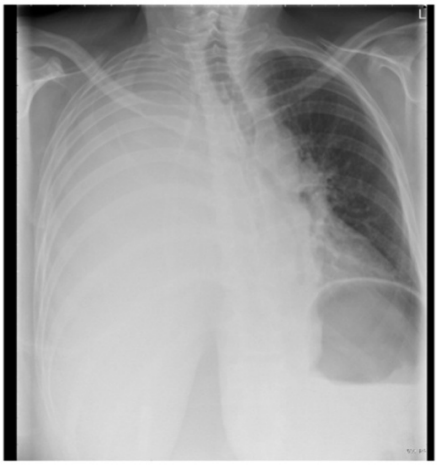

Figure 1: Chest X-ray demonstrates pleural effusion.

Intraoperative findings were multiple lesions in the lung parenchyma and diffuse bleeding from multiple small abnormal nodules on the surface of the lung and chest wall. Despite transfusion with blood products (thrombocytes, red blood cells) during the next days, there was still ongoing bleeding and on seventh postoperative day, the patient underwent re-thoracotomy in order to achieve hemostasis. Intraoperative findings were less diffuse bleeding from lung and chest wall than at the primary operation and the hemostasis was achieved.

Biopsies from multiple sites from the lung and chest wall were sent for histological investigation. Microscopically the material was made of disorganized fibromyxoid tissue with massive bleeding and some necrosis. In areas of preserved tissue there were irregular vascular or cystic structures or solid angulated groups of malignant cells. The tumour cells were large with plump or polygonal nuclei with vesicular chromatin pattern and prominent nucleoli (Figure 2).

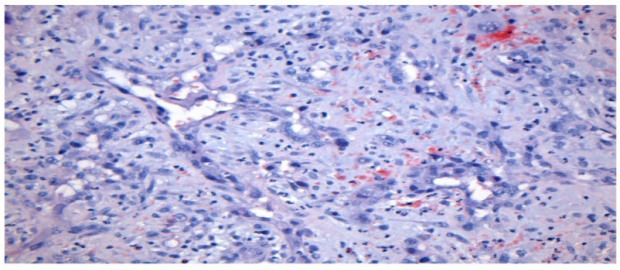

Figure 2: The figure demonstrates irregular vascular structures and diffuse infiltrating cells with variation in size and atypical nuclei (Hematoxylin-eosin x200).

In a broad panel of immunohistochemical markers the tumour cells were negative for epithelial, mesothelial and melanocytic antibodies. There was positive staining for vimentin, CD31 (Figure 3).

Figure 3: The figure demonstrates intraluminal proliferation and solid groups of cells with positive staining for CD31 (x100)

Von Willenbrand Factor VIII, and partly for podoplanin (D2-40) and WT1, but was negative. The pattern was high lightened by staining for collagen IV.

The vascular pattern with intraluminal proliferations of malignant cells together with immunohistochemical positivity for endothelial markers made the diagnosis of angiosarcoma and with the primary tumour burden in the lung parenchyma, primary pulmonary angiosarcoma.

DISCUSSION

In this rare case the patient presented with a hemothorax, which is most often caused either by chest trauma, iatrogenic event or intrathoracic carcinoma. Hemothorax should be suspected in hypovolemic patients with chest X-ray demonstrating pleural effusion. Blood loss in pleural cavity may be massive and even minor injury can lead to significant blood loss.

Only 0.7% of all malignant tumours are sarcomas. Angiosarcoma (AS) is a malignant tumour of vascular endothelial origin contributing with only one percent of all sarcomas.[1-5] The presence of AS in the lung is usually a consequence of metastasis from another primary site.[1-8] The most common primary sites for AS are the skin (60%), soft tissue (25%) and in the visceral organs predominantly the heart, liver and spleen. In English spoken literature there have only been reported a few cases describing primary pulmonary angiosarcoma.[2]

AS in the lung can be uninodular involving one vessel or airway or multifocal with ill-defined, soft, red nodules in the lungs and pleura[3,7,9,10] as it was demonstrated perioperative in this case. Primary pulmonary angiosarcoma is characterized by insidious growth, where the tumour usually demonstrates

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extensive local invasion and haematogenous metastases by the time of presentation[2,4,8] therefore curative intended treatment is not an option and the treatment of choice is palliative therapy.

The prognosis for angiosarcoma is in general poor and the survival rate range from 1-9 months after initial diagnosis.[1-3-,9-11] Only patients with surgically resectable primary sarcomas of the extremities, breast, head and neck have long-term survivals while patients presenting with primary sarcomas in respectively thorax and abdomen or recurrent AS and metastatic AS have poor survival.[8]

Different therapeutic modalities have been attempted, but none of them have shown to change the course of the disease.[1,5,11,12]

The few reported primary pulmonary AS all presented with bleeding either as intractable haemoptysis, massive intraparenchymal bleeding or intrapleural haemorrhage.[3] The symptoms are unspecific but described as chest pain, haemoptysis, dyspnoea, cough, and malaise.[5,7,9,10,12] At the primary operation the priority was to achieve haemostasis that went successful but after seven days we had to do re-thoracotomy in order to achieve haemostasis again. The disease was too widespread in the lung and at the chest wall at that time accordingly curative radical surgery was not an option. Her poor general condition did that she died one day after discharge from the hospital.

CONCLUSION

Primary pulmonary angiosarcoma has a poor prognosis in itself but can also cause serious and life-threating intractable intrathoracic bleeding.

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Case Report

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Flapping Tremor as a Diagnostic Tool for Evaluation of Hypercapnia

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ABSTRACT

A 76 year-old woman was admitted to our hospital because of aspiration pneumonia. Four days after initiation of the antibiotic treatment, her respiratory status became to be better; however, hypoxemia was recurred with tachypnea (36 breaths/min). On physical examination, she had a hot hand and flapping tremor, but not loss of consciousness, suggesting of acute type 2 respiratory failures with the increased level of PCO_2 ranged from 15 to 30 mmHg. Accumulation of the evidence showed that the PCO_2 retention from the usual level for each patient could be assessed by physical sings. Thus, even in the modern era, general physicians can easily diagnose the rapid increase the level of PCO_2 from the prior status by using the physical findings such as hot hands, flapping tremor, and loss of consciousness as in the present case.

KEYWORDS: Flapping tremor; Hypercapnia; Hot hand.

BACKGROUND

The diagnosis of acute type 2 respiratory failure needs a multidisciplinary assessment. Among them, physical diagnosis would be a pivotal role for rapid and precise diagnosis

CASE REPORT

A 76 year-old woman was admitted to our hospital with a chief complaint of dyspnea (day 1). She had primary Sjogren's syndrome with pulmonary involvement (usual interstitial pneumonia) two years previously, and underwent home oxygen therapy for recent three months (only at effort 4 L/min). On admission, vital signs were as follows, blood pressure of 180/100 mmHg, heart rate of 120 beats/min, respiratory rate of 24 breaths/min, body temperature of 37.1°C, and SpO_2 of 84% with a 4 L/min oxygen supply *via* nasal mask. Her consciousness was clear, and physical examination showed coarse crackles in the right lung field and fine crackles in throughout the lungs. Based on the diagnosis of aspiration pneumonia, she was treated with ampicillin/sulbactam 3 g q6h, and her condition was improved even at nasal cannula of 1 L/min at day 3. However, at day 4, hypoxemia was recurred with tachypnea (36 breaths/min) together with hot hand and flapping tremor, suggesting of emergence of acute type 2 respiratory failure.

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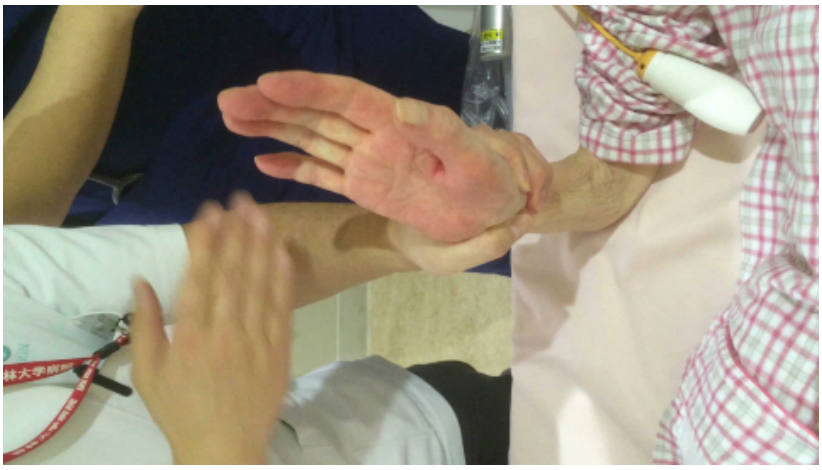
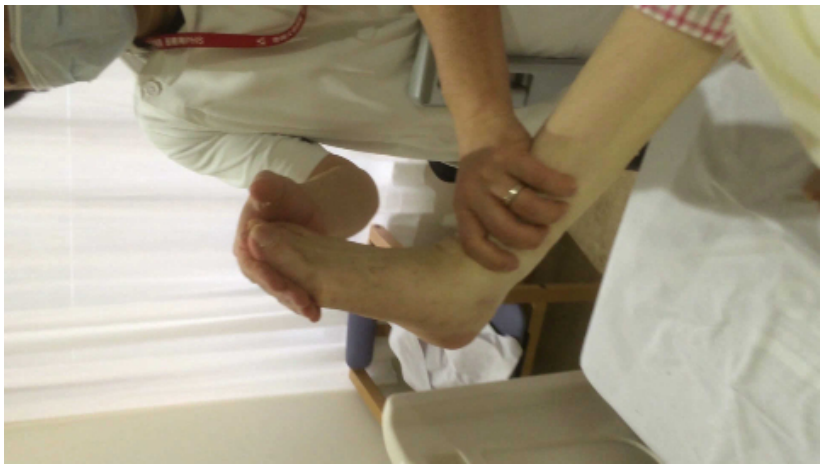


Video 1: The flapping tremor at right hand of the patient.



Video 2: The flapping tremor at right foot of the patient.

Indeed, the present case showed that the increase of $PaCO_2$ at day 4 from the baseline (3months before) was 20.3 mmHg, which was confirmed by the advent of hot hand and flapping tremor, but not loss of consciousness (Table 1). Thereafter, regardless of the initiation of non-invasive positive pressure ventilation, she died of acute type 2 respiratory failure at day 14.

DISSCUSION

Hypercapnia can cause hot hand, flapping tremor, and loss of consciousness with an increase of $PaCO_2$ from the prior status at 5 mmHg, 15 mmHg, and 30 mmHg, respectively,[1] as in the present case. Although Gross et al.[1] reported that the different signs of hypercapnia to the carbon dioxide were assessed by mixed venous CO_2 using Campbell and Howell rebreathing methods,[2] those signs were present in the cases of CO_2 narcosis using the $PaCO_2$ instead of mixed venous CO_2.[3] Furthermore, accumulation of the evidence showed that the PCO_2 retention from the usual level for each patient could be assessed by following signs such as hot hand (≥5 Torr), a rapid bounding pulse or small pupils (≥10 Torr), engorged fundal veins or flapping tremor (≥15 Torr), confusion or drowsiness (≥30 Torr), and coma (≥50 Torr).[1-3] Thus, whenever physicians encounter the patients with acute type 2 respiratory failure at their initial visits, physical examination would be a clue to the diagnosis for assessing the increase from the prior status in $PaCO_2$.

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Time	3 Months before	On admission	Day 4	Day 5
Oxygen supply	room air	nasal 4L/min	nasal 3L/min	venturimask 31%
Hot hand	-	-	+	+
Flapping tremor	-	-	+	-
Loss of consciousness	-	-	-	-
pH	7.379	7.442	7.382	7.431
pCO_2(mmHg)	41.4	46	61.7	52.7
pO_2(mmHg)	87.4	59.7	54.6	51.3
HCO_3- (mEq/L)	23.9	30.9	35.8	34.5

Table 1: Clinical findings at each point.

CONCLUSIONS

The presence of hot hand, flapping tremor, and loss of consciousness are useful and easy way of assessment for the elevation of $PaCO_2$ from the prior status.

CONFLICTS OF INTEREST: None

PATIENT CONSENT

This report has no personally identifiable information, and informed consent was obtained from the patient.

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Research

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Role of MicroRNAs in Progression and Recurrence of Early-Stage Lung Adenocarcinoma

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ABSTRACT

Lung cancer is the leading cause of cancer-related death worldwide, and the majority of cases (77%) are not diagnosed until the disease has spread to regional lymph nodes or distant sites. Even among Non-Small Cell Lung Cancer (NSCLC) adenocarcinoma patients who have been diagnosed early and where there has been no spread to lymph nodes, recurrence after surgical intervention is high. Improved understanding of the molecular alterations involved in aggressivity and recurrence of these tumors may provide better biomarkers for the identification of patients who would benefit from adjuvant chemotherapy. By comparing the expression of microRNAs in advanced Stage II/III tumors with those expressed in earlier Stage I tumors, we aimed to identify differentially expressed molecular biomarkers that could underly progression and recurrence of Stage I tumors. This pilot study utilized TaqMan qPCR assays to assess the expression of microRNAs in tumor tissue, matched normal tissue and plasma samples from Stage I and Stage II/III lung adenocarcinoma patients. Seven microRNAs were identified from plasma that could distinguish between patients with Stage I and Stage II/III adenocarcinoma. The up-regulation of miR-29a in plasma of patients with later-stage adenocarcinoma would result in enhanced expression of several molecules involved in integrin signaling, migration and proliferation. Analysis of differential expression of microRNAs in later-stage compared to early-stage lung adenocarcinoma implicates focal adhesion and ECM-receptor pathways in progression and recurrence. Plasma miR-29a is a promising biomarker that can be assessed non-invasively and whose clinical utility should be pursued.

KEYWORDS: Adenocarcinoma; Biomarker; microRNAs; Non-Small Cell Lung Cancer; Recurrence; Transcriptome.

ABBREVIATIONS: ECM: Extracellular Matrix; KEGG: Kyoto Encyclopedia of Genes and Genomes; MAPK: Mitogen-Activated Protein Kinase; NSCLC: Non-Small Cell Lung Cancer; qPCR: quantitative Polymerase Chain Reaction; RNA: Ribonucleic Acid.

Note: All gene names are abbreviated according to the Human Genome Nomenclature Committee.

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INTRODUCTION

Lung cancer remains the leading cause of cancer-related death worldwide, accounting for 1.59 million of the 8.2 million total cancer deaths each year.[1] NSCLC accounts for ~80% of all lung cancers, and adenocarcinoma is the main histological subtype. Smoking is responsible for 87% of lung cancers in the United States, and the majority of NSCLC adenocarcinomas. While historically, cigarette smoking was associated with NSCLC squamous cell lung cancer, since the transition to filtered cigarettes there has been a rise in the number of adeocarcinomas among smokers. Most NSCLC patients are diagnosed at an advanced stage that is associated with high recurrence and less than 15% survival over 5 years. Even for patients with Stage I disease who have had a complete surgical resection, the 5-year survival is only 52%,[2] mostly because of recurrence. This suggests that Stage I tumors can be misclassified, especially when lymph node metastases are small and escape detection. Furthermore, intratumor heterogeneity may not be detected by standard single-site biopsy, leading to inaccurate classification and prognosis.[3,4] Classification and staging by Tumor, Node, Metastasis (TNM)[5] have only modest prognostic utility and patients with resected Stage IA tumors of identical histology, differentiation, vascular invasion, and margins may differ widely in their survival time and response to therapy. The use of alternative sources of biopsy material that reflect tumor heterogeneity, such as readily available blood or plasma, may provide valuable information.

Cells and cellular nucleic acids in sputum, bronchial biopsies, brushing specimens, bronchial lavage fluid, blood, pleural effusions and solid tumor biopsies provide material for classification, diagnosis and prognosis of NSCLC.[6] Several cellular tumor markers have been shown to be useful in prognosis of lung cancer including positive immunohistochemical staining of mTOR in early stage NSCLC,[7] overexpression of Apolipoprotein E in lung adenocarcinomas with malignant pleural effusion,[8] and overexpression of SOX2 in Stage I adenocarcinomas, especially those with pleural invasion.[9] Recently, a 14-gene expression assay has been developed to examine recurrence risk in early-stage NSCLC.[10]

Small non-coding RNAs such as snoRNAs[11] and microRNAs ("oncomirs")[12] are a promising class of cancer biomarkers. The expression profiles of these highly stable molecules in tumor tissue and/or plasma have been associated with occurrence of NSCLC, tumor tissue type, stage of differentiation, and prognosis (for review see[13]). microRNAs are generally down-regulated in tumor tissue compared to matched normal tissue. However, tumor-derived microRNAs are found within exosomes or in free circulation in the plasma of cancer patients[14] and have been shown to be generally up-regulated compared to plasma from normal donors.[15] A panel of four plasma microRNAs (mir-486, -30d, -1 and -499) was recently shown to discriminate between NSCLC patients with good and poor prognosis.[15] Serum levels of miR-142-3p and miR-29b were elevated in lung adenocarcinoma patients suffering recurrence within 24 months.[16]

There is an urgent need for better understanding of the molecular alterations that occur during progression of early-stage lung adenocarcinoma, and more reliable prognostic and predictive biomarkers. Such biomarkers could improve survival by identifying patients at high risk for recurrence who may benefit from adjuvant chemotherapy. Here we report a pilot study that determines microRNA profiles in tumors and plasma from patients with Stage I and Stage II/III adenocarcinoma, the most common form of NSCLC, and relate these findings to the biological processes that may be involved in early recurrence.

MATERIALS AND METHODS

Study population

Patient biopsy samples (frozen tumor and plasma) from Stage I (with no lymph node involvement) and Stage II/III (with lymph node involvement) were selected from the Lung Cancer Tumor Bank at Capital District Health Authority (CDHA; Halifax, NS, Canada). In order to limit the clinical variability as much as possible, a uniform study population was used based on the following criteria: adenocarcinoma, age >45 years; all but one were current or past smokers (Table 1).

ID	Stage	Age	Gender	Smoking Status	Recurrence (months)
L202	IB	76	Male	Current	
L218	IA	65	Male	Past	
L247	IB	77	Male	Past	
L252	IA	81	Male	Past	
L272	IA	79	Female	Past	
L278	IA	69	Male	Past	
L194	IIA	53	Male	Past	12.8
L212	IIA	71	Male	Current	
L229	IIA	66	Male	Past	8.9
L240	IIA	74	Male	Past	
L258	IIIA	45	Male	Never	
L262	IIA	76	Female	Past	
L300[a]	IIB	54	Female	Past	

[a]removed from analyses due to high hemolysis in plasma samples.

Table 1: Patient characteristics and samples used for microRNA analysis.

Most cases of adenocarcinoma are associated with smoking, and these were highly represented in the Tumor Bank. Recurrence is the time in months from surgery until detection of recurrence. Control plasma (Precision Biologics, Dartmouth, NS, Canada) contained pooled plasma from 20 healthy subjects. This study was approved by the Capital Health Research Ethics Board (CDHA-RS/2004-336), and all participating individuals signed informed consent.

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RNA extraction

Approximately 50 mg of frozen lung tissue was pulverized in a MultiSample BioPulverizer (BioSpec, Bartlesville, OK, USA) and homogenized in 1 mL TRIZOL® (Life Technologies, Burlington, ON, Canada) using a FastPrep®-24 (MP Biomedicals, Santa Ana, CA, USA). Total RNA was extracted according to the manufacturer's protocol and treated with TURBO DNase (Applied Biosystems, Carlsbad, CA, USA) prior to further purification with the Total RNA Purification Kit (Norgen Biotech Corp., Thorold, ON, Canada). RNA was purified from 300 µL of plasma using the Total RNA Purification Kit and both preparations were enriched for small RNAs using the PureLink miRNA Isolation Kit (Invitrogen, Burlington, ON, Canada). The RNA was quantified on the NanoDrop-1000 (NanoDrop Products, Wilmington, DE, USA) and RNA quality was determined on the Bioanalyzer-2100 (Agilent, Wilmington, DE, USA).

MicroRNA assay of tissue and plasma

The miRNA PCR Array for Cancer (MAH-102F; SA Biosciences, Burlington, ON, Canada) containing 88 cancer-relevant microRNA-probes and 8 controls in a 96-well plate was employed to assess expression of microRNAs in pooled plasma samples of five Stage I (L202, 218, 247, 252 and 278) and five Stage II (194, 212, 229, 240 and 262) lung adenocarcinoma patients compared to pooled normal plasma (Precision Biologics). RNA was purified from 300 µL of plasma (60 µL each of five individual samples from each stage). 200 ng enriched small RNA was reverse-transcribed using the RT² First Strand Kit (SA Biosciences, Burlington, ON, Canada) and qPCR was performed on a Light Cycler-480 (Roche Applied Science, Laval, QC, Canada) using conditions specified by the manufacturer. Fold regulation was calculated from 2nd derivative Cp values using the SA Biosciences web-based qPCR analysis software (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php) and SNORD48 was used as a reference.

Based on the results of the PCR array (Supp Table 1) and literature survey, a multiplex TaqMan® microRNA Assay (Applied Biosystems) was designed for 31 microRNAs that were at least two-fold up-regulated in cancer relative to control (Supp Table 2). RNU48 was used as a reference, allowing all 32 reactions to be performed in triplicate in 96-well plates. Expression was evaluated in an expanded group of six Stage I and seven Stage II/III tissue and plasma samples. Briefly, 100 ng of total RNA was reverse-transcribed using the TaqMan® microRNA Reverse Transcription Kit (Applied Biosystems) and qPCR reactions were cycled on a LightCycler-480 once at 95 °C for 10 min; 45 times at 95 °C for 15 sec, 60 °C for 60 sec; and once at 40 °C for 30 sec. Individual samples contained 1 µL cDNA, 5 µL of 2X qPCR mix and 1 µL of microRNA-specific TaqMan Probe/Primer mix in a 10 µL total reaction volume. For tumor samples, matched normal tissue samples in triplicate were used as controls and for plasma, pooled normal samples were used. In the latter case, control samples were assayed twice independently in triplicate reactions and the average was used.

Normalized data was analyzed by Student's t-test and p-values were based on replicate 2nd derivative Cp values for each microRNA in the control group and patient group. Those microRNAs that were associated with high p-values (>0.05) were assigned as missing values. The Student's t-test and SAM modules of MeV[17] were then used to identify microRNAs that were significantly differentially expressed between the Stage I and Stage II/III samples. MicroRNA expression was analyzed from 2nd derivative Cp values using SA Bioscience web-based PCR data analysis software (Qiagen, Mississauga, ON, Canada) and the stably expressed RNU48 and miR-210 to normalize for tissue and plasma samples, respectively. Targets for microRNAs were identified by searching miRDB (www.mirDB.org). The list of microRNA targets with a score greater than 80 was used to identify KEGG pathways potentially involved in progression using gene enrichment analysis in WebGestalt (http://bioinfo.vanderbilt.edu/webgestalt).

RESULTS

In order to identify clinically relevant microRNA biomarkers, we screened 88 cancer-relevant microRNAs by array. We selected those that were up-regulated at least two-fold in plasma of lung adenocarcinoma patients relative to plasma from pooled normal subjects as these would be most easily detectable in a clinical assay. Sixty-eight microRNAs were up-regulated in plasma of NSCLC patients relative to normal subjects. Of these 44 microRNAs showed a higher level in Stage II/III *vs.* Stage I, 17 showed a lower level in Stage II/III *vs.* Stage I, and 7 did not differ between Stage II/III and Stage I (Table 2).

Of these, 20 for which there was corroborating evidence in the literature were chosen for TaqMan assays along with 11 other well-supported microRNAs from the literature (miR-17-3p, 34a, 92a, 106a, 141, 182, 201, 218, 221, 451 and 486-5p; Supp Table 2).

Expression data for these 31 microRNAs were obtained using TaqMan assays for all tissue/matched normal samples and all but one plasma sample (L300 plasma was unusable due to a high level of lysed erythrocytes and was excluded from analysis). Several microRNAs (miR-17-3p, 141, 143, 205 and 218) exhibited inconsistent, often low, signal intensities in most plasma samples (grey boxes, Figure 1).

microRNAs were mostly down-regulated in tumor samples relative to normal adjacent tissue but up-regulated in plasma relative to control pooled plasma from normal subjects (Figure 1, Supp Table 3) . The single Stage IIIA sample (L258) exhibited generally higher, but not statistically significant, up-regulation of plasma microRNAs than the other samples.

There were no statistically significant differences in tumor tissue microRNA expression between different stages.

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Up-regulated in plasma of patients relative to normal			Down-regulated or same relative to normal
Up Stg II/III *vs*. Stg I	**Down StgII/III *vs*. StgI**	**Same Stg2II/III *vs*. StgI**	
let-7g	let-7a	let-7d	miR-122
let-7i	let-7b	miR-124	miR-125a-5p
miR-1	let-7c	miR-127-5p	miR-125b
miR-100	let-7e	miR-146b-5p	miR-133b
miR-126	let-7f	miR-23b	miR-134
miR-130a	miR-10a	miR-25	miR-135b
miR-132	miR-10b	miR-373	miR-149
miR-140-5p	miR-128		miR-150
miR-142-5p	miR-138		miR-184
miR-143	miR-155		miR-18b
miR-144	miR-15b		miR-193b
miR-146a	miR-183		miR-196a
miR-148a	miR-199a-3p		miR-196a
miR-148b	miR-200c		miR-206
miR-15a	miR-21		miR-210
miR-16	miR-7		miR-212
miR-17	miR-98		miR-218
miR-181a			miR-34c-5p
miR-181b			miR-9
miR-181c			miR-92a
miR-181d			
miR-18a			
miR-191			
miR-193a-5p			
miR-19a			
miR-203			
miR-205			
miR-20a			
miR-20b			
miR-214			
miR-215			
miR-222			
miR-27a			
miR-27b			
miR-29a			
miR-29b			
miR-301a			
miR-30c			
miR-32			
miR-335			
miR-363			
miR-372			
miR-378			
miR-96			

Table 2: Expression of 88 cancer-relevant miRNAs in plasma of NSCLC Stage I and Stage II/III patients relative to normal plasma, determined by PCR Array. MicroRNAs that are shaded were included in the TaqMan assay.

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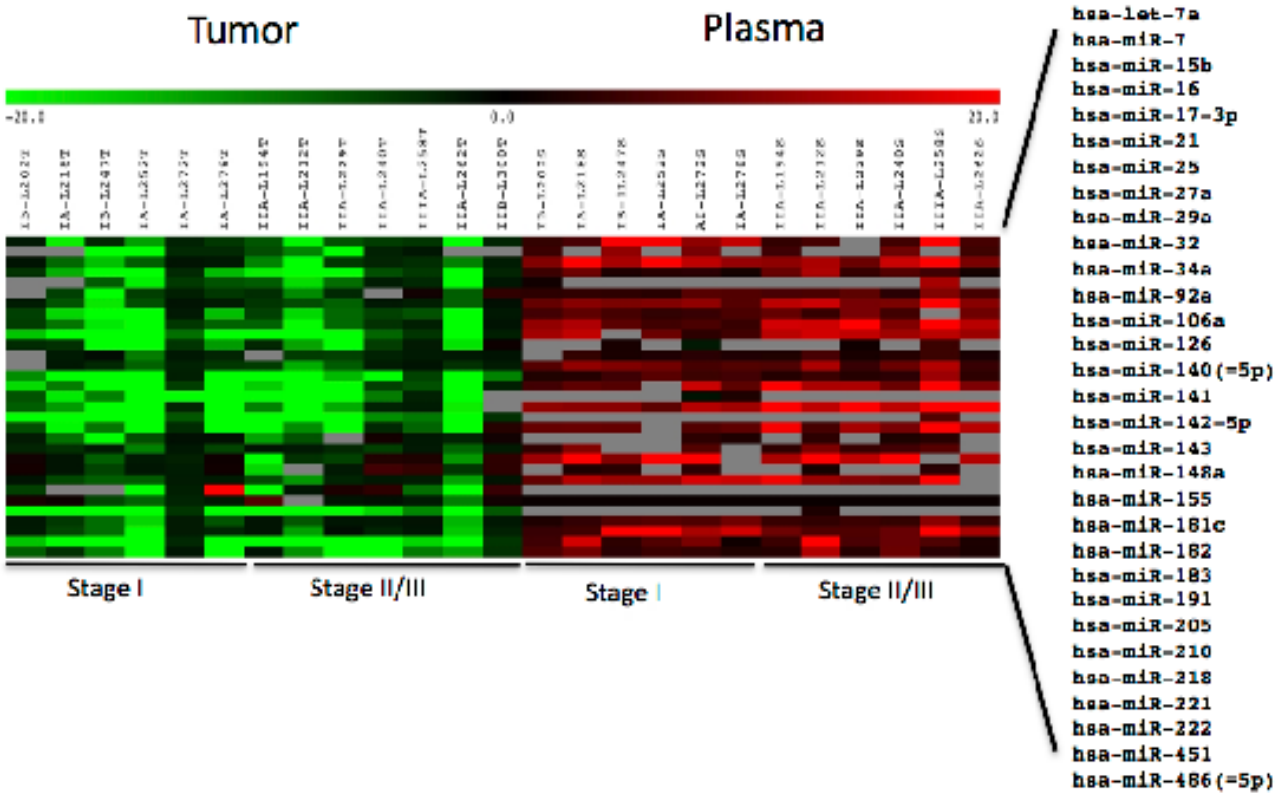
However, SAM analysis identified seven plasma microRNAs that could distinguish between patients with Stage I and Stage II/III lung adenocarcinoma (Figure 2).

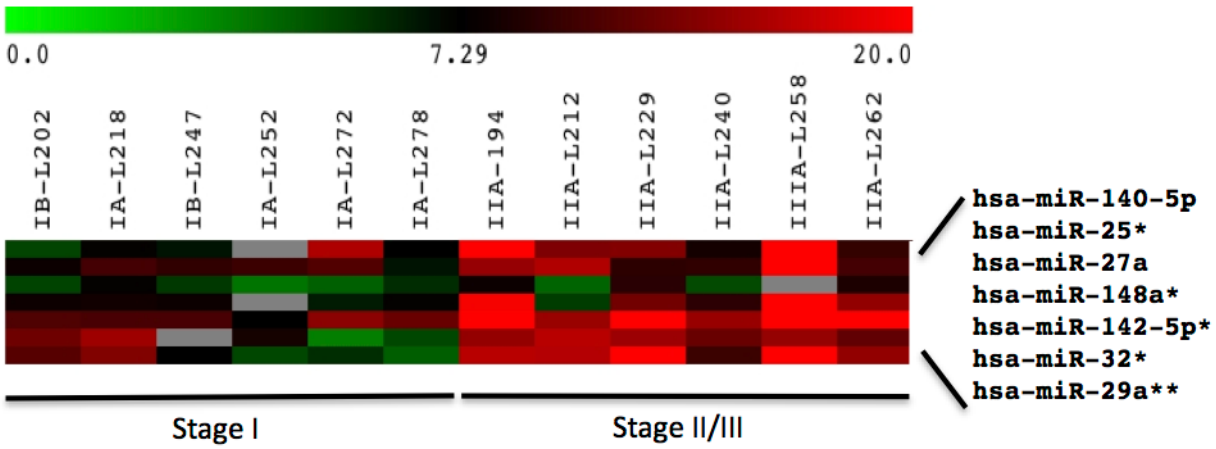
Four of these (miR-25, 32, 142-5p, and 148a) approached significance ($p<0.1$) and miR-29a was identified at $p<0.01$ by t-test (Table 3).

Searching miRDB for targets of these seven microRNAs that were up-regulated in Stage II/III compared to Stage I patients resulted in 416 unique targets with a score greater than 80 in the human genome (Supp Table 4). Enrichment analysis of these targets identified five major KEGG pathways: cancer, focal adhesion, ECM-receptor, p53 signalling, and MAPK signaling pathway (Supp Table 5). The highest-scoring cancer-relevant targets are shown in Table 3.



Expression of 31 miRNAs in tumor relative to matched normal tissue and patient plasma relative to pooled control plasma from six patients each with Stage I and Stage II/III NSCLC adenocarcinoma. Red, up-regulated; green, down-regulated; grey, high p-values (>0.05) were assigned as missing data. Log2 ratios are presented.

Figure 1: Heat map of miRNA expression in tumor tissue.



Significantly differentially expressed plasma miRNAs identified by SAM analysis. Those marked with a single asterisk or double asterisk were also identified as significant by t-test ($p<0.1$ and $p<0.01$, respectively). Red, up-regulated; green, down-regulated; grey, high p-values (>0.05) were assigned as missing data. Log2 ratios are presented.

Figure 2: Heat map of miRNA expression in plasma.

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microRNA	Score	Gene Name	Description	Function in Cancer
miR-29a**	100	TET3	Tet oncogene family member 3	n/a
	99	COL3A1	Collagen, type III, alpha 1	Invasion
	98	HBP1	HMG-box transcription factor	Invasion
	95	TET2	Tet oncogene family member 2	n/a
	93	IGF1	Insulin-like growth factor 1	Proliferation
	91	COL11A1	Collagen, type XI, alpha 1	Invasion
	91	FBN1	Fibrillin 1	Invasion
	89	COL4A5	Collagen, type IV, alpha 5	Invasion
	88	COL4A4	Collagen, type IV, alpha 4	Invasion
miR-32*	100	CD69	CD69 receptor for NK cells	Anti-tumor immunity
	96	FBXW7	F-box and WD repeat domain containing 7	Proliferation
	95	RAB23	RAB23, RAS oncogene family	Invasion
	93	MYO1B	Myosin 1B	Invasion
miR-25*	98	CD69	CD69 receptor for NK cells	Anti-tumor immunity
	97	RAB23	RAB23, RAS oncogene family	Invasion
	92	MYO1B	Myosin 1B	Invasion
	90	FBXW7	F-box and WD repeat domain containing 7	Proliferation
miR-142-5p*	100	MEGF1	Multiple EGF-like domains 10 (FAT2)	Invasion
	100	ITGAV	Integrin alpha V	Invasion
	98	BAI3	Brain-specific angiogenesis inhibitor 3	n/a
	96	CHSY3	Chondroitin sulfate synthase	Proliferation
	96	PSAT1	Phosphoserine aminotransferase 1	Proliferation
	93	IGF2BP3	IGF2 mRNA binding protein	Proliferation
miR-148a*	96	EIF2C4	Eukaryotic translation initiation factor 2C, 4	Invasion
	96	YWHAB	Tyrosine 3-monooxygenase activation protein beta	Proliferation
	91	FLT1	Fms-related tyrosine kinase 1	Invasion
miR-27a	100	GXYLT1	Glucoside xylosyltransferase 1	Cell communication
	99	PLK2	Polo-like kinase 2	Proliferation
	96	SNAP25	Synaptosomal-associated protein, 25	n/a
	96	FBXW7	F-box and WD repeat domain containing 7	Proliferation
	96	PPARG	Peroxisome proliferator-activated receptor gamma	Proliferation
	95	ST6GAL NAC3	ST6 N-acetylgalactosaminide alpha-2,6-sialyltrans-ferase 3	Invasion, proliferation
	93	GALNT7	UDP-N-acetyl-alpha-D galactosamine:polypeptide N acetylgalactosaminyltransferase 7	Invasion, proliferation
miR-140-5p	97	FGF9	Fibroblast growth factor 9	Proliferation
	91	SEPT2	Septin2	Invasion
	89	FBN1	Fibrillin 1	Invasion

Table 3: Cancer-relevant targets of plasma microRNAs that distinguish between Stage I and Stage II/III tumors. microRNAs were identified using SAM and those marked with a single or double asterisk were significant by t-Test ($p<0.1$ and $p<0.01$, respectively). Targets were identified by searching miRDB. n/a, no direct role in NSCLC.

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DISCUSSION

In this study, we found a consistent down-regulation of microRNAs in tumor compared to adjacent normal tissue and up-regulation of plasma microRNAs from lung cancer patients relative to normal controls. Of the 31 microRNAs included in the TaqMan assay, most were consistently detectable; however five microRNAs had highly variable, often low, values between triplicates, giving a low p-value. Two of these, miR-17-3p and 205, have been reported to have very weak intensities in plasma and serum.[18] There was a slightly lower overall expression of tumor microRNAs and corresponding higher overall expression of plasma microRNAs for the youngest patient (L258), but this was not statistically significant. This patient was also the only non-smoker and had the most advanced stage (IIIA). Although biological features of the patient can impact microRNA levels, it would be difficult to draw any valid conclusions concerning microRNA dysregulation based on these features from the results of a single patient.

We were unable to detect any statistically significant changes in tumor tissue microRNAs between Stage I and Stage II/III patients. However, patient plasma did exhibit several microRNAs (miR-25, 27, 29a, 32, 140-5p, 142-5p, and 148a) that could distinguish between patients with Stage I and Stage II/III lung adenocarcinoma, with miR-29a being the most statistically significant ($p<0.01$). Since plasma is a more easily accessible biopsy material, plasma profiles rather than tissue profiles were used to investigate alterations in cancer pathways.

There is limited information on the expression of these microRNAs and their target genes in other solid tumor types. There have, however, been some studies describing these microRNAs as prognostic biomarkers and others describing the same genes targeted by the dysregulated microRNAs we have identified. These studies mainly include tumors associated with the digestive system (particularly colorectal) and lend support to our findings (Table 4).

Similar to lung adenocarcinoma, expression of miR-29a is also down-regulated in gastric tumors[19] and oral squamous carcinoma,[20] resulting in increased expression of Matrix Metalloproteinase[2] (MMP2) in the latter case. MMP2 degrades extracellular matrix and promotes tumor invasiveness. In Stage II colorectal cancer, down-regulated tissue miR-29a is associated with high recurrence[21] and elevated plasma levels are associated with advanced stage.[22] Consistent with our results, the miR-29 family has been reported to be down-regulated in NSCLC tumors relative to normal tissue[23,24] and up-regulated in serum of early stage lung adenocarcinoma patients who recurred within 24 months.[16] We also found miR-142-5p to be up-regulated in plasma of later-stage patients. This microRNA is derived from the same precursor as miR-142-3p, which is significantly up-regulated in serum of early stage lung adenocarcinoma patients who recurred within 24 months16 and in plasma of patients with aggressive NSCLC.[25]

Of the gene targets of differentially regulated microRNAs listed in Table 3, several do not have a known direct role in NSCLC and are not discussed. These include Brain-specific Angiogenesis Inhibitor (BAI3), SNAP25 (involved in neurotransmitter release) and TET2 and TET3 oncogenes (epigenetic regu-

microRNA	Gene target	Function	Tumor type	Reference
miR-29a	nd	Invasion	Gastric	21
	MMP2	Invasion, apoptosis	Oral squamous	22
	nd	Prognosis	Colorectal	23
	nd	Early detection	Colorectal	24
	COL11A1	Progression	Gastric	28
	COL11A1	Cancer-associated stromal cells	Colorectal	29
miR-142-5p	MEGF (FAT2)	Invasion	Squamous carcinoma	35
	PSAT	Proliferation	Colorectal	36
	IGF2BP3	Prognosis	Colorectal	38
	IGF2BP3	Prognosis	Pancreatic ductal	40
	CHSY3	Invasion	Colorectal	39
miR-148a	MMP7	Invasion	Gastric	21
	EIF2C4	Metastasis	Colorectal	42
miR-27a	GALNT7*	Invasion, proliferation	Cervical	44
miR-140-5p	SEPT2	Proliferation	Liver	47

Table 4: microRNAs dysregulated in this study that have been reported to be prognostic or have targets that function in other cancer types. Only in the case of miR-29a and MMP2 (shaded) have the microRNA and target gene been demonstrated to be co-regulated in the other tumor types. *GALNT7 regulation in cervical cancer is associated with miR-214 expression. nd, not determined.

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lators in malignant hematopoiesis).[26]

The remaining gene targets have roles in ECM-receptor interactions, focal adhesion, cell motility and migration, angiogenesis, protein modification, invasion, proliferation, cell signalling and NK-cell anti-tumor immunity (Figure 3).

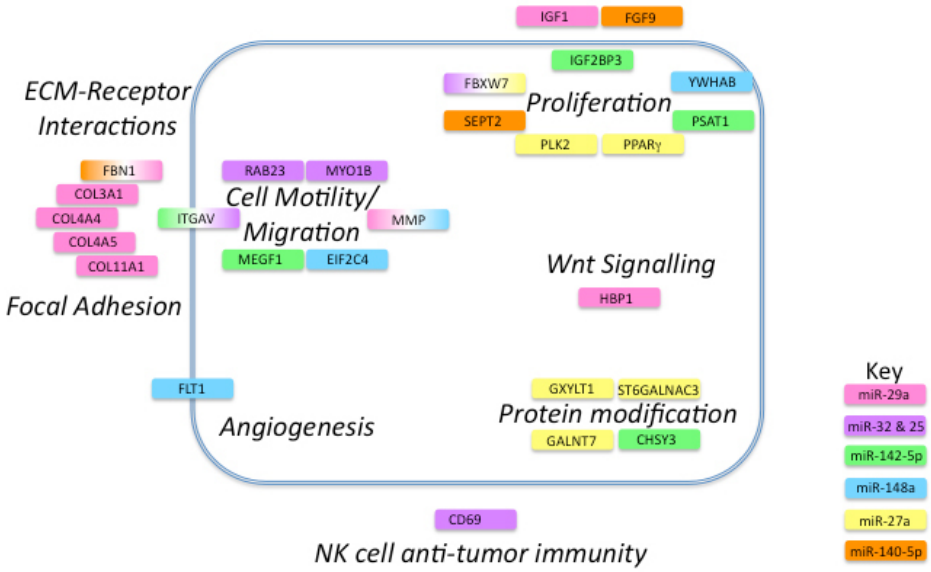
This is in agreement with a recent microarray study we performed on the same tumor biopsies[27] in which ECM-receptor interactions and components of the focal adhesion pathway were the main elements differentially regulated between early and later-stage lung adenocarcinomas.

miR-29a targets collagens COL3A1, COL11A1, COL4A5, and COL4A4, and Fibrillin1 (FBN1), which participate in integrin signaling, focal adhesion and migration. In gastric cancer, transcripts for several collagens, including COLA11A1, are also up-regulated in malignant compared to pre-malignant tumors[28] and COLA11A1 is also up-regulated in cancer-associated fibroblasts of colon adenocarcinomas.[29] Insulin-like Growth Factor 1 (IGF1) is a key player in NSCLC tumorigenesis and metastasis,[30] initiating cell signaling, promoting mitosis, proliferation and differentiation, and inhibiting apoptosis. HBP1 is a transcriptional repressor that plays a role in Wnt signaling and induces cellular transformation.[31] Reduction in tumor tissue miR-29a would result in enhanced IGF-1 and Wnt signalling.

Interestingly, we found two microRNAs (miR-25 and miR-32) that both targeted the same four genes (CD69, FBXW7, RAB23 and MYO1B). As with any biomarker discovery, there is high potential for false positives due to the large number of variables relative to the number of patient samples. Therefore, this overlap in targets suggests that these microRNAs and their target transcripts warrant further investigation as biomarkers in early lung adenocarcinoma. Their higher expression in plasma and concomitant low expression in tumor tissue would promote translation of the hedgehog-signalling factor, RAB23 and the cytoskeletal protein MYO1B, which are necessary for cell motility and metastasis.[32] FBXW7 is part of the E3 ubiquitin ligase complex and targets cyclin E for degradation, thus impacting cell proliferation.

microRNAs in the plasma can affect immune cell function. CD69 is a marker of NK-cell activation and a positive regulator of anti-tumor activity.[33] It has been shown that administration of several synthetic microRNAs or microRNAs in exosomes from healthy donors to NK-cells and mice bearing tumors resulted in TLR1 activation and increased NF-kB signaling. This resulted in increased NK-cell activation via up-regulation of CD69 and increased anti-tumor immunity.[34] However, high plasma levels of miR-32 and -25, which repress CD69 expression, would result in reduced NK-cell anti-tumor activity leading to tumor progression.

miR-142-5p targets MEGF1 (FAT2), which mediates migration of human cutaneous squamous carcinoma cells,[35] and PSAT1, which has been shown to stimulate proliferation in colorectal cancer.[36] miR-142-5p also targets ITGAV and its reduction in tumor tissue would result in an increase in ITGAV. Interestingly, miR-32 and miR-25 also target ITGAV[37] and miR-32 is reported to be down-regulated in another lung cancer study.[23] CHSY3 and IGF2BP3 are two other relevant targets of miR-142-5p and both are over-expressed in colorectal cancer patients relative to healthy controls.[38,39] CHSY3 is responsible for sulfation



Gene targets are represented by colored boxes with colors indicating microRNA involved. If the gene is targeted by more than one microRNA a gradient of the appropriate color is applied. The tumor cell membrane is represented by a blue line and targets are shown as extracellular, intracellular or membrane-bound. Names of pathways are shown in bold italic font.

Figure 3: Cancer pathways affected by microRNAs differentially regulated in NSCLC progression.

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of glycosaminoglycans on the cell surface and shows elevated expression in colorectal cancer;[39] IGF2BP3 has been suggested as a biomarker of poor prognosis in pancreatic ductal adenocarcinoma.[40]

miR-148a, like miR-29a, is down-regulated in gastric cancer and has a major impact on the expression of MMP7 and invasiveness.[19] miR-148a also targets FLT1, the receptor for Vascular Endothelial Growth Factor (VEGFR1) and thus could play an important role in tumor angiogenesis. Decreased levels of tumor miR-148a would result in higher levels of FLT1 available for angiogenic signaling. NSCLC tumors expressing FLT1 are more malignant and associated with poorer outcomes.[41] Similarly, higher levels of YWHAB, a 14-3-3 protein involved in cell cycle control and inhibition of apoptosis, would result in a more malignant phenotype. EIF2C4 is an argonaute protein in the RNA-induced silencing complex. Argonaute proteins are up-regulated in colon cancer and higher levels are associated with distant metastasis.[42]

Two other microRNAs that showed statistically significant differential expression by SAM (but not t-test) target key genes of importance in cancer progression. miR-27a regulates expression of three enzymes that participate in post-secondary modification of important proteins (GXYLT1, GALNT7, ST6GALNAC3). GXYLT1 glycosylates NOTCH proteins and reduces cancer signalling[43] whereas GALNT7 and ST6GALNAC3 glycosylate mucins, thus impacting proliferation, migration, and invasion. Oncogenesis in cervical cancer cells is effected through miR-214 regulation of GALNT7.[44] PPAR-γ is a nuclear hormone receptor that is involved in cell proliferation and is over-expressed in many human cancers. It has recently been shown to prevent metastasis in lung cancer cells by inhibiting TGF-β induced epithelial-to-mesenchymal transition.[45] PLK2 is a serine-threonine protein kinase that is essential for cell division.[46] miR-140-5p, like miR-29a, targets FBN1. SEPT2, which plays an important role regulation of cell proliferation through its effect on actin filament formation and has been suggested as a target for liver cancer therapy,[47] is also a target of miR-140-5p. The growth factor FGF9 is also involved in cell proliferation and can synergize with the EGFR oncogenic pathway in lung adenocarcinoma48 leading to recurrence.[49]

The use of microRNAs as clinical biomarkers is increasingly being described in the literature.[50] They are highly stable in circulating blood plasma and can be readily isolated. They are tissue-specific and their expression can be dysregulated in response to physiological changes such as disease. Furthermore, they play a key role in differentiation, proliferation and apoptosis making them ideal diagnostic, prognostic and predictive biomarkers in cancer. Although there are some published studies describing microRNA signatures associated with recurrence in lung cancer, they tend to include lung cancer patients with multiple NSCLC tumor types and from multiple stages.[15,51,52] It is well known that different types of NSCLC (such as squamous and adenocarcinoma) express different miRNAs. In our small-scale study, we have restricted our comparison to only adenocarcinomas of Stage I and Stage II/III in order to more accurately identify plasma microRNAs likely to be involved in recurrence in early-stage patients. Of the six Stage I and six Stage II/III patients studied, recurrences only occurred in two patients both of whom were Stage II.

CONCLUSIONS

In conclusion, our study indicates that several microRNAs that distinguish between Stage I and Stage II/III patients are involved in the regulation of important pathways in cancer biology including focal adhesion, ECM-receptor interactions, p53 signalling, and MAPK signaling pathway. Because of the small number of samples in this study, their role in lung adenocarcinoma should be verified in a larger patient cohort. Their presence in plasma provides a readily accessible biofluid for clinical testing and the significant up-regulation of miR-29a indicates it could be a promising biomarker.

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Review

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Tuberculosis and Pregnancy: An Updated Systematic Review

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ABSTRACT

Tuberculosis (TB) affects women, especially in the child-bearing years. TB is associated with a poorer outcome of pregnancy, although this may be due to the general risk factors for TB, namely poverty, malnutrition and overcrowding. New studies have shown that symptom screening has a low sensitivity and specificity, but is improved by the addition of a tuberculin skin test (TST) or interferon-gamma release assay (IGRA) or, in high incidence areas, DNA amplification tests (e.g. Xpert MTB/RIF). TB-HIV co-infection is a common cause of mortality and morbidity in pregnancy. The diagnostic process remains the same in pregnancy, but non-specific symptoms and extra pulmonary disease demand a higher level of suspicion of TB. Standard first-line treatment is safe in pregnancy. Data on second-line drugs in pregnancy is still limited, but injectable drugs may affect the hearing and balance of the fetus. The IGRA responses appear to change during pregnancy, with more positive responses after delivery. The increasing incidence of drug-resistant TB, especially in Eastern Europe and Central Asia, requires an evaluation of the safety of second-line drugs in pregnancy.

KEYWORDS: Tuberclosis; Pregnancy; Mycobacterium tuberculosis; Maternal mortality.

ABBREVIATIONS: TB: Tuberculosis; TST: Tuberculin Skin Test; MeSH: Medical Subject Heading; IGRA: Interferon-Gamma Release Assays; PLHIV: People Living with HIV; QFT: QuantiFERON Gold-in-Tube; BCG: Bacille Calmette-Guérin; CXR: Chest X-Ray; LTBI: Latent Tuberculosis Infection.

INTRODUCTION

Tuberculosis (TB) remains an important disease, despite effective treatment for the last 50 years. The number of cases remains high at 8.6 million new cases and 1.3 million TB deaths (WHO, 2012).[1] Although TB affects men more commonly than women, there were still 2.9 million cases and 410,000 deaths among women, predominantly in the 15-44 year age group, which coincides with the age of childbearing.[1] In 2011, there were an estimated 216,500 (95% uncertainty range 192,100 to 247,000) active tuberculosis cases in pregnant women.[2] Pregnancy itself appears to be a risk factor for developing TB.[3] The increased susceptibility to TB may be due to immunological changes in pregnancy. Pregnancy partially suppresses the T-helper 1 (Th1) cell mediated immunity, in favour of the antibody response (Th2 mediated), perhaps to protect the fetus from immunological rejection.[3] Cell-mediated immunity has the dominant role in protection against *Mycobacterium tuberculosis* and active TB is associated with a dominant Th2 immune response.[3]

This review updates our knowledge about TB in pregnancy, with particular reference to studies since January 2012.

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METHODS

The biomedical databases MEDLINE, through the search engine PubMed, and EMBASE (Elsevier) were searched. The time limit was May 2014 - Jan 2012. The Medical Subject Heading (MeSH) terms "tuberculosis", "pregnancy", "maternal mortality" and "women's health" were combined as a major topic in PubMed MEDLINE. A common search strategy was used for all databases, employing a combination of the following terms: "pregnancy", "maternal", "tuberculosis", "congenital", "latent tuberculosis infection", "multidrug resistant tuberculosis". The research was limited to English-language articles and human studies. Case reports and conference abstracts were excluded. Titles and abstracts were reviewed and irrelevant topics were excluded. To complete the search a manual search was made of bibliographic references cited in the original papers included. These articles were then contextualised within current knowledge of TB in pregnancy.

RESULTS

Maternal tuberculosis and pregnancy outcomes

TB disease in pregnancy is associated with adverse pregnancy outcomes.[4] Toxaemia (pre-eclampsia), vaginal bleeding, fetal death at 16-28 weeks, acute fetal distress, prematurity (<37 weeks), small for date, low birth weight (< 2.5 kg), and perinatal death have all been described, but are also associated with poverty, malnutrition and overcrowding - factors themselves associated with TB.[3,4] Adverse perinatal outcomes have been associated with incomplete treatment, delayed diagnosis and advanced pulmonary involvement.[4] A new case-control study conducted in UK showed a lower birth weight in infants born to mothers with TB, especially if pulmonary disease, despite receiving standard treatment.[5] Maternal TB is a risk factor for child TB. Congenital TB is rare,[6] but the risk of transmission to the infant in the postpartum period is higher due to inhalation of aerial droplets coughed out by the mother. One study in South Africa detected TB in 16% of neonates born to mothers with suspected or proven TB.[7] In Kenya, infants with early TB infection (T-SPOT.*TB* positivity in 6 month old children born from HIV positive mothers) had a 15.5-fold increased odds of having a mother with active TB (P = 0.04).[8]

TB and ectopic pregnancy

An association between acute ectopic gestation and genital tuberculosis has been suggested. In a group of 17 adolescents with acute presentation of ectopic pregnancy, 6 out of 17 (35, 29%) had genital tuberculosis compared with 5% in the control groups (1 out of 20, p=0.03).[9]

Symptom screening active TB in pregnant women

A four symptom screening (cough, fever, night sweat and weight loss) has been proposed by the WHO as a first step in finding TB in people living with HIV (PLHIV).[10] A meta-analysis estimated a sensitivity of 79% and specificity of 50% for these symptoms.[11] However, when tested in 799 pregnant women from India, the sensitivity was 54.5%, and only reached 100% if combined with a tuberculin skin test (TST).[12] A much larger study in South Africa (1415 pregnant women) demonstrated that the WHO four-symptom screen failed to identify most cases of TB in HIV-positive pregnant women[13] as most (73%) were asymptomatic. The sensitivity of having any one of the four symptoms for TB disease was 28%, giving a specificity of 84%, positive predictive value 4.4% and a negative predictive value of 98%. Cough, irrespective of duration, was the most sensitive of the symptoms (23%). In Kenya, screening for the four symptoms in HIV-positive pregnant women was not helpful: 3/26 (12%) symptomatic patients had an abnormal chest X-ray (CXR) consistent with TB, while 7/100 asymptomatic pregnant women who had a CXR were then treated for TB.[14] The same group screened 2980 HIV-positive and HIV-negative pregnant women, 17 of whom were treated for TB on the basis of clinical judgment and/or CXR positivity but just 4 had a one of the following features: a cough of >2 weeks, bloody cough in the past year, fever of >3 weeks, past history of TB diagnosis, history of TB contact in the household, weight loss/failure to gain weight if pregnant in the past year.[15]

The poor performance of symptom screening suggests that additional tests are needed to intensify the effective case finding in HIV pregnant women.

The performance of Tuberculin Skin Test (TST) and Interferon-Gamma Release Assays (IGRAs) in pregnancy

Pregnancy does not appear to alter the performance of the TST.[16] Tests based on the interferon-γ response to proteins that are not found in *Mycobacterium tuberculosis*-Bacille Calmette-Guérin (BCG), were designed to improve the specificity of the diagnosis of TB infection.[17] A comparison of TST and QuantiFERON Gold-in-Tube (QFT) in 199 pregnant women at a US public hospital showed a greater specificity for the IGRA, in that BCG vaccination at birth was an independent predictor of a positive TST but not a positive IGRA.[18] Pregnancy itself appeared not to affect the likelihood of a positive QFT, comparing 140 pregnant and 140 non-pregnant adolescents and young women, and exposure to TB correlated better with the IGRA than with TST.[19] However, there were twice as many indeterminate results due to a low mitogen response in the pregnant compared with the non-pregnant cohort. By contrast, in India, HIV-negative pregnant women, presenting to the antenatal clinic in their late second or third trimester, showed more positive QFT tests than positive TSTs (37% and 14% respectively), even though 5 TU and a cut-off of 10 mm for TST positivity was used as in the US studies.[20]

A cross-sectional study suggested that a positive QFT

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was more likely in the postpartum period, but attempts to perform a cohort study on 60 women were thwarted by the failure of follow-up, such that the initial finding could not be confirmed.[20]

HIV-TB co-infection in pregnancy

The estimated percentage of TB cases living with HIV remains at 13% globally and the African Region accounts for 75% of the estimated number of HIV-positive incident TB cases.[1] Pregnant women with HIV infection have a higher risk of developing active TB than HIV-negative pregnant women.[21,22] TB increases maternal mortality in HIV co-infection.[3,4] An analysis of pregnancy-related mortality in western Kenya found that nearly two-thirds of deaths were due to indirect, non-obstetric causes and HIV/AIDS and TB accounted for 45% and 10% respectively of these deaths.[23] In a rural sub-district in South Africa from 1992 to 2010, the obstetric mortality ratio averaged 185 per 100,000 live births and deaths during pregnancy were 423 per 100,000 live births, the difference being primarily due to HIV/AIDS and pulmonary TB.[24] Furthermore, mothers with HIV co-infection have a higher rate of infecting their children (30%) compared to those with TB alone (12%).[25]

IGRA in HIV-positive pregnant women

One group in Kenya has examined the value of the IGRA T-SPOT. *TB* using cryopreserved peripheral blood mononuclear in a cohort of 327 HIV-positive pregnant women in Kenya.[26,27,28] In the first report, 36% of the HIV-infected women were IGRA-positive during pregnancy, which likely reflects the local rate of LTBI. In multivariate analysis, adjusting for baseline CD4 count, IGRA positivity was associated with a 4.5 (1.1-18.0)-fold increased risk of active TB (p=0.03). Maternal and infant mortality was higher in mothers with a positive IGRA but the difference was not statistically different unless the maternal CD4 count was < 250 cells/mm^3.[26] Eighteen had a positive test and the number of spot counts was highest at 32 weeks, then fell around delivery, followed by a later increase.[27] Nine (3%) women developed TB within 1 year of childbirth, 6/110 (6%) with a positive test and 3/148 (2%) with a negative test at 32 weeks of pregnancy (no significant difference; indeterminate results were found in 52, of whom 8 had <20 spot counts in the positive control). The prognostic sensitivity and specificity improved if the CD4 count was <250 mm^3, although a positive T-SPOT.*TB* test was more common in those with a higher CD4 count.[28]

A cost-effectiveness analysis of IGRA in HIV-positive pregnant women in low TB incidence countries found that testing with TB-SPOT.TB alone was the most cost-effective strategy where the incidence of TB was ≥1.25%, but if the incidence of TB was less than 1,250 per 100,000, screening with TST and then testing with the T-SPOT.*TB* test was better.[29]

Screening for Latent Tuberculosis Infection (LTBI)

The WHO has recommended isoniazid preventive treatment therapy for all HIV infected individuals, including pregnant women.[10] The US Centre for Disease Control and Prevention (CDC) recommend screening for LTBI only in high risk women, i.e. those with known or suspected tuberculosis contact, injection drug use, HIV or other immune suppression, foreign birth and residence in communal settings.[30] Antenatal care is an ideal opportunity to identify pregnant women with LTBI, as it is often the first time healthcare has been accessed, especially in these high risk groups that might find accessing healthcare difficult.[3,31] In high-burden countries, the social conditions of the woman often limit access to healthcare and antenatal care should be integrated with TB screening and treatment.[32]

Isoniazid preventive treatment

Isoniazid is safe in pregnancy and is not teratogenic.[33] Unlike rifampicin, there are no interactions between isoniazid and antiretroviral treatment.[34] A WHO guideline has recommended 6 months preventive treatment on the basis of studies available to them.[10] Clinical trials have suggested an increased benefit by increasing isoniazid preventive treatment to 36 months duration in PLHIV, although none reached statistical significance.[35,36,37] The safety of long-term isoniazid prophylaxis was examined in HIV-infected women who were pregnant during the course of therapy and no adverse pregnancy outcomes were observed compared to the control group.[38]

TB diagnosis in pregnancy

There is no difference in the diagnostic approach between pregnant and non-pregnant women. Sputum examination and a CXR are the most important investigations. Concern about radiation safety in pregnancy has limited the use of chest radiography, but shielding the abdomen and the lower doses of radiation that are now required to obtain a CXR mean that the exposure to the fetus is considered negligible.[39] However, the symptoms of TB are non-specific and are commonly present during normal pregnancy e.g. general malaise, fatigue, appetite loss; extrapulmonary disease, which is more difficult to detect, is also more common in pregnancy.[3,4]

Xpert® MTB/RIF, a PCR test on sputum that detects the presence of *M. tuberculosis* DNA and genetic mutations that indicate resistance to rifampicin, has been proposed as an instrument for active pulmonary case finding[40] and in antenatal clinics in a high TB burden setting.[41] In Zambia, sputum samples from 94 patients admitted with a primary obstetric or gynaecological problem (67% pregnant or < 6 weeks post natal patients and 74% HIV infected patients) were analysed by sputum smear microscopy, culture and Xpert® MTB/RIF assay. Among the participants, 26 had culture-confirmed TB (77% in pregnant or postpartum women). In these circumstances, Xpert® had a sensitivity of 81% and a specificity of 97% compared to sputum culture and was more sensitive than sputum smear microscopy alone (50%).[42]

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TB treatment in pregnancy

The standard first line treatment for pulmonary and extrapulmonary TB does not differ between pregnant and non-pregnant women. The WHO recommend 2 months of isoniazid, rifampicin, pyrazinamide, and ethambutol, followed by 4 months of isoniazid and rifampicin.[43] This regimen is safe to use during pregnancy.[33] Anti-TB therapy should not be a reason to discontinue breastfeeding.[33] Pyridoxine supplementation is recommended for all pregnant or breastfeeding women taking isoniazid. Streptomycin is contraindicated during pregnancy because of damage to the eighth cranial nerve with ototoxicity.[33]

Second line treatment in pregnancy

Multidrug-resistant TB (MDRTB) is defined as TB caused by organisms resistant to isoniazid and rifampicin; extensively drug-resistant TB (XDRTB) is defined as MDRTB resistant as well to any one of the fluoroquinolones and to at least one of three injectable second-line drugs.[44] Globally, in 2012, an estimated 450,000 people developed MDRTB resulting in 170,000 deaths. The highest levels of MDRTB are found in Eastern Europe and Central Asia. In the treatment of MDRTB, WHO advises giving at least four drugs that are known or likely to be effective against the drug-resistant *M. tuberculosis* strain isolated, plus pyrazinamide. If possible, WHO group 2 (amikacin, capreomycin or kanamycin) and WHO group 3 (fluoroquinolones) should be core drugs with the rest (etionamide/protionamide and cycloserine) being accompanying drugs.[44] New drugs, bedaquiline, an oral diarylquinoline which inhibits the proton pump ATP synthase, and delamanid, which is a nitro-dihydro-imidazooxazole derivative with mycobacteria-specific antibacterial activity *in vitro* that inhibit mycolic acid biosynthesis, have been approved for use in the USA since December 2012 and EU since April 2014 respectively, but their use in pregnancy needs evaluation.[45,46]

Although second-line drugs are used during pregnancy, little is known about the safety of these drugs for the fetus and about the outcome in MDRTB cases during pregnancy. The largest study to our knowledge is of 38 pregnant women treated for MDRTB in Peru and outcomes were similar to those of the general local population.[47] The majority of the second-line drugs are in FDA class C (animal studies suggest a problem, but human studies are inadequate), except for aminoglycosides, which are in class D (definite evidence of fetal risk). A TBNET consensus statement on the management of patients with M/XDRTB in Europe states that "safe treatment of M/XDRTB during pregnancy seems possible but needs individual decision-making" and "Pregnancies should not be terminated because of M/XDR-TB";"Aminoglycosides/polypeptides are not recommended for M/XDRTB treatment during pregnancy"; "Patients should be advised to maintain double barrier contraception during treatment of M/XDRTB".[48]

CONCLUSION

There is a need for cohort studies of IGRA in pregnancy in women without HIV to assess the consistency of these tests and any changes that might occur as pregnancy proceeds. The increasing incidence of drug-resistant TB, especially in Eastern Europe and Central Asia, requires an evaluation of the safety of second-line drugs in pregnancy.

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Review

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Pulmonary Complications in Patients with Brain Injury

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SUMMARY

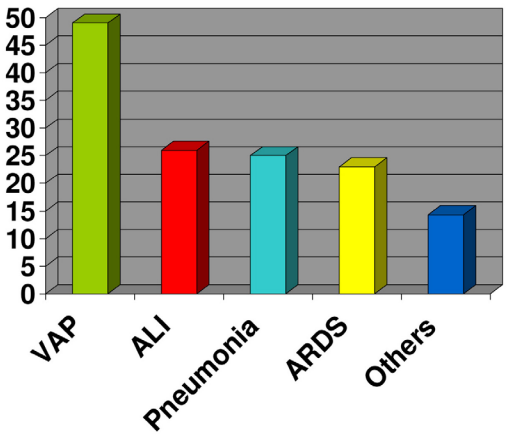
Critically ill patients with brain injury associated with organ dysfunction among which include pulmonary involvement as a determinant of morbidity and mortality. The aim of this paper is to review the major complications associated with brain injury in patients with brain injury, etiology, clinical, prevention and treatment.

KEYWORDS: Brain injury; Pulmonary complications; Patients with brain injury.

ABBREVIATIONS: CNS: Central Nervous System; NPE: Neurogenic Pulmonary Edema; SAH: Subarachnoid hemorrhage; ICP: Intracranial pressure; ACI: Acute Lung Injury; ARDS: Acute Respiratory Distress Syndrome.

INTRODUCTION

The severity of brain injury is the main determinant of morbidity and mortality in neurocritical patients. However, the role of other associated extracranial complications should not be disregarded[1] with pulmonary complications being among the most common ones (Figure 1).[2] Major respiratory complications associated with injury to the Central Nervous System (CNS) are caused by airways dysfunction (inability to maintain passage due to neurologic depression or damage), respiratory muscles dysfunction (nerve injury) or intrinsic pulmonary disorders (infection, embolism, acute respiratory distress syndrome, etc.). The occurrence of such complications could potentially cause hypoxemia, which would secondarily aggravate the brain damage. This situation is known to account for up to 50% deaths resulting from brain injury and is considered to be an independent factor for mortality.[1,3-8]



VAP: Ventilator-Associated Pneumonia; ALI: Acute Lung Injury; ARSD: Adult Respiratory Distress Syndrome

Figure 1: Extracerebral complications in neurocritics patient.

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The objective of the present article was to offer an up to date review of theories and available evidence on relation between the cerebral alterations and the interactions to pulmonary level.

PATHOPHYSIOLOGY

The interaction between the CNS and the respiratory system is evidenced at the physiological level since the respiratory center, located in the brain stem, controls involuntary respiratory activity and a second, less defined, center in the brain controls for the voluntary respiratory activity.

The ventilatory activity of airways and chest muscles is triggered by spinal cord and carotid sinuses, which are in turn stimulated by CO_2 and Hydrogen ion [H+] concentrations. Carotid sinuses also respond to changes in PaO_2. Current theories proposed to explain the pathophysiological mechanisms of respiratory complications in case of brain injury are summarized as follows (Figure 2).

Sympathetic Storm

When brain injury occurs, an initial sympathetic discharge elevates plasmatic adrenaline levels to about 1200 times the normal value within seconds. Although the level of adrenaline subsequently falls, it keeps 3 times higher than normal for about ten days.[2-3,7,9-10] This catecholamine release elevates intravascular pressure thus damaging the endothelium and producing pulmonary edema due to disruption of the alveolar-capillary barrier. The initial hydrostatic edema becomes a protein-rich edema that goes into interstitial and alveolar spaces.[3,7,9-10] The amount of fluid that leaks through the endothelium depends on the severity of capillary hypertension. Furthermore, in case of structural damage to the capillary wall, plasma will pass to the interstitial and alveolar spaces. The medulla oblongata is considered to be the key anatomical structure directly involved in this pathophysiological response; experiments demonstrated that bilateral lesions to this structure cause systemic hypertension and lung injury.[11] Additionally, the possible involvement of the hypothalamus (dysfunction of the vasomotor center), elevated Intracranial pressure (ICP) or activation of the sympathetic-adrenal system have also been studied.[11]

Inflammatory Theory

As part of the inflammatory response to acute brain injury, intracranial production of cytokines may increase and the permeability of the blood-brain barrier may be higher. The resulting transcranial release of pro-inflammatory cytokines could cause migration of neutrophils and activation of macrophages in the alveolar spaces, as well as structural damage to type II pneumocytes, thus producing secondary lung damage.[12-18]

Double Injury Theory (Double Hit Model)

Brain injury would produce a systemic inflammatory response, which would increase vulnerability to inflammatory processes well tolerated in normal situations, such as infection, mechanical stress from artificial ventilation or surgical procedures. Such inflammatory processes could in turn worsen the initial brain damage, harm distant organs and cause multiorgan

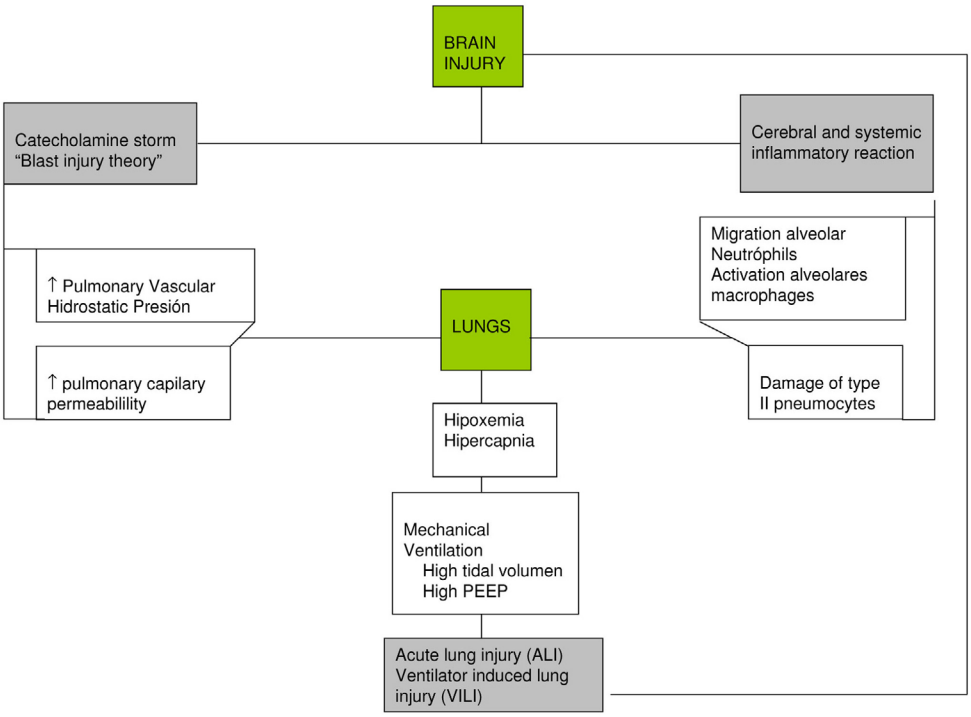

Figure 2: Pathophysiology of lung complications in patients with brain injury.

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dysfunction.[19-23]

After severe brain injury, respiratory failure is the commonest associated dysfunction. In such cases, pathophysiological processes to be considered are: preclinical lung injury, altered capillary permeability with activated neutrophils and macrophages migrating into the airways and alveolar spaces, increased concentrations of pro-inflammatory cytokines in bronchoalveolar lavage and damage to type II pneumocytes - characterized by intense cell vacuolation and lipid peroxidation of lung tissue that causes lysis of the cell membranes. All of this would make lungs more susceptible to secondary damage ("double lesion model") that could result from initial trauma, respiratory strategy, infection, transfusion, etc.[19-23]

These experimental and clinical data support the hypothesis that after severe brain injury eventually leading to brain death, preclinical lung injury occurs. The catecholamine storm and the systemic production of inflammatory mediators create a systemic inflammatory environment where the lung is more susceptible to further injurious stimuli, such as ventilatory settings, infections, and transfusions.

CLINICAL PICTURE

Neurogenic Pulmonary Edema (NPE)

Neurogenic Pulmonary Edema is a complication of severe CNS injury that develops early after trauma in the absence of prior cardiac or pulmonary dysfunction. It was first described in 1908 by Shanahan,[24] who reported the finding of pulmonary edema in 11 patients with epilepsy. It is occasionally classified as a type of acute respiratory distress, although its pathophysiology and prognosis are different (Table 1). Its severity is related to the magnitude of the brain injury and it is associated with high morbidity rates and up to 7% mortality.[8,10,25] NPE may be triggered by different brain conditions, including seizures, stroke and traumatic brain injury (Figure 3). NPE is most common in seizures during the post-critical period, affecting to up to one third of patients in status epilepticus.[26-27]

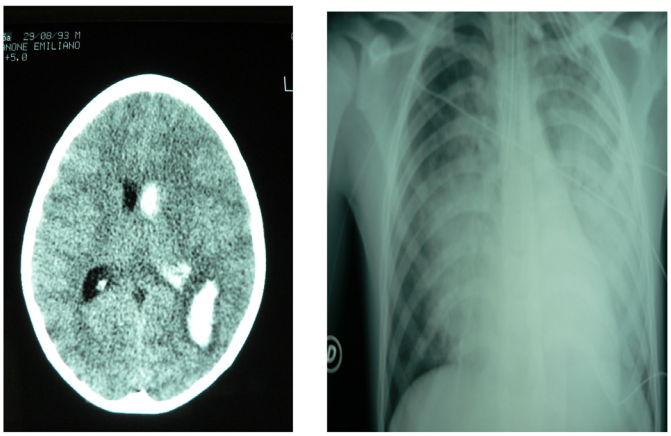

Figure 3: Neurogenic pulmonary edema associated with intracelebral homorrhage.

	↑ Hydrostatic Pressure	↑ Permeability
Cardiogenic pulmonary edema	++++	
Reexpansion pulmonary edema	++++	++
Neonatal respiratory distress syndrome	++	++++
Neurogenic pulmonary edema	++++	++++
High altitude pulmonary edema	++++	++
Adult respiratory distress syndrome / Acute lung injury		++++
Negative pressure pulmonary edema	++++	++++

Table 1: Pathophysiology types of lung edema.

Among patients with brain hemorrhage, NPE mostly occurs in those with Subarachnoid hemorrhage (SAH) from ruptured aneurysms and is correlated with the clinical and radiological severity of bleeding.[24-26]

In head injury the development of pulmonary edema associated with intracranial hypertension %), although not necessary, with a lower incidence (around 1%).[26] Clinical presentation of NPE typically comprises early onset and rapid development during the first few hours or days after brain injury; it usually resolves within 24-48 hours. Its most common symptom is dyspnea, accompanied by tachypnea, tachycardia, and crackles at baseline. It should be diagnosed in the presence of pulmonary edema and after excluding other possible causes. NPE diagnosis is different from that of other types of pulmonary edema (Table 1).

Changes in the Ventilation/Perfusion Ratio

Described in neurocritical patients with hypoxemia in the absence of pulmonary infiltrate (clinical-X ray dissociation), probably related to an alteration in the ventilation/perfusion ratio. It is caused by redistribution of regional perfusion, pulmonary microembolism with increased dead space and removal of pulmonary surfactants due to excessive sympathetic stimulation and hyperventilation.

Acute Lung Injury/Acute Respiratory Distress Syndrome

The occurrence and severity of Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS) in patients with severe brain damage mainly depend on: the systemic inflammatory response associated with the release of mediators (cytokines, interleukins, tissue factors, etc.),[14-18] the massive release of catecholamines into the bloodstream resulting from brain damage and the resuscitation maneuvers that have been

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used.[3,10] All of this increases lung capillary permeability and results in bilateral pulmonary infiltrates that lead to severe hypoxemia with a low PaO_2/FiO_2 ratio (<300 mm Hg in ALI, <200 mm Hg in ARDS) and decreased lung compliance.

ALI/ARDS occurs in between 20-25% of patients with severe brain damage and constitutes an independent risk factor that worsens prognosis and increases mortality in these patients. Although direct correlation with the CT-scan images of lesions has not been demonstrated, evidence indicates that patients with the larger, more severe brain injury (larger evacuated mass or displacements from midline) are at 5 to 10 times higher risk of developing ALI.[3,7]

ALI/ARDS develops relatively soon with an initial peak 2-3 days after starting mechanical ventilation or late at the week.

Risk factors for developing ALI/ARDS include: structural damage observable in initial brain CT-scan study, early detection of low Glasgow Coma Score (GCS), use of vasoactive drugs and history of drug addiction.[3,7,28]

Pneumonia Associated with Mechanical Ventilation

Mechanical Ventilation-Associated Pneumonia (VAP) is defined as pneumonia developed 48-72 hours after intubation. Many patients with severe brain damage require artificial respiratory support, often prolonged, with the consequent risk of lung infection and prolonged stay in the Intensive Care Unit. The incidence of VAP is 9-27% of all intubated patients and increases with the duration of mechanical ventilation. It is even higher in neurocritical patients, where variable incidence rates oscillate between 30% and 50%.[29] VAP mostly appears during the first 4 days. The most commonly involved pathogens are: *Pseudomona aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Acinetobacter*, although in patients with head trauma *Staphylococcus aureus* is the most common one.[30] Independent risk factors for the development of VAP are: low level of consciousness, aspiration, emergency intubation, mechanical ventilation for more than 3 days, reintubation, age over 60 years, supine position, comorbidities and previous antibiotic therapy.[30]

Mortality rates attributed to VAP are about 30%; this condition doubles the risk of death. It is also estimated to increases the duration of stay at the ICU in 6 days and the associated expenses in 10,000 dollars.[30]

Mechanical ventilation is the main supportive therapy used to re-establish sufficient oxygen supply and remove Carbon dioxide (CO_2) produced by peripheral organs during acute respiratory failure in patients with severe brain injury. While tight CO_2 control represents a priority in patients with severe brain injury, optimal treatment of ALI/ARDS consists of a protective ventilatory strategy allowing a certain degree of hypercapnia to protect the lung from further injurious stimuli while it recovers from the initial pathological process.

All these findings support the hypothesis that, after severe brain injury eventually leading to brain death, a preclinical lung injury characterized by an inflammatory response is present. Massive brain injury may act as a preconditioning factor rendering the lung more susceptible to subsequent lung damage induced by mechanical ventilation.

PREVENTION AND TREATMENT OF PULMONARY COMPLICATIONS

Associated with Brain Injury

Management of Neurogenic Pulmonary Edema (NPE)

Neurocritical patients with impaired respiratory function or signs of low cardiac output require strict hemodynamic and echocardiographic monitoring. These patients may potentially need a pulmonary artery catheter (Swan-Ganz). Fluid restriction as normal filling pressure is reached and use beta blockers to reduce oxygen consumption (VO_2) may be necessary to improve lung and brain function. Vasopressor agents should be used rationally to avoid possible vasoconstriction of pulmonary vessels or worsening of lung injury due to hemodynamic stress. Respiratory support with supplemental oxygen should be provided with the aim of maintaining SaO_2> 94%. Mechanical ventilation should be indicated if necessary.[27] The respiratory strategy should include alveolar recruitment and protection in order to avoid ventilation-induced injury (see below).

Prevention of Collapse /Atelectasis in Decline Areas

Mechanical ventilation with tidal volumes of 6 ml/kg, plateau pressure less than 30 cm H_2O and PEEP less than 15 cm H_2O ensures homogenization of pulmonary ventilation preventing lung collapse that avoid hemodynamic variations which impact on Cerebral Perfusion Pressure (CPP). It has been shown that use of high tidal volumes (9-11 ml/kg) stimulates inflammatory response and lung injury induced by the ventilator.[10,25,31-32]

Even neurocritical patient's ventilation in prone possition results in improved brain tissue oxygenation in severe hypoxemia with minimal effect on Intracranial pressure (ICP) and Cerebral Perfusion Pressure (CPP).[25,33-36]

Prevention of Mechanical Ventilation-Associated

Pneumonia

Prolonged respiratory support should be avoided; mechanical ventilation should be discontinued as soon as possible.[32,34] However, in cases of neurocritical disease prompted, patients that require high doses of sedatives or failure to protect the airways (alteration of respiratory center, damage to the nuclei of cranial nerves or ARDS), a tracheostomy may improve

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patient's comfort and management of tracheobronchial secretions (Table 2), as well as early onset of chest physiotherapy.[36]

- Neurologic stability
- Normal Intracraneal pressure
- Weaning posibility
- Pronlonged alteration of consciousness
- Imposibility to protect airways
- Easier management of pulmonary secretions
- Obstruction of airways

Table 2: Indications for tracheostomy in neurocritic patients.

Prophylactic antibiotic therapy against multiresistant pathogens is usually started until the microbiology laboratory results are available. The treatment usually lasts for 7 days if the patient progresses favourably; although it might be extended in patients where *Pseudomonas aeruginosa* is identified since infection by this organism is associated with high rates of relapse.[37]

CONCLUSIONS

In patients with severe brain damage, respiratory complications are frequent. Increasing our knowledge of the pathophysiologic mechanisms triggering this situation is essential to enhance prevention and treatment. Key elements to be highlighted include worsening of brain injury secondary to hypoxia and lung damage, and aggravation of lung injury due to inadequate respiratory strategy.

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