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USO DE MARCADORES MOLECULARES EM AMOSTRAS OBTIDAS DE PUNÇÃO ASPIRATIVA PRÉ-OPERATÓRIA DE TIREOIDE: ANÁLISE SECUNDÁRIA DE DADOS

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Tese apresentada ao Programa de Pós-graduação em Medicina e Saúde, da Faculdade de Medicina da Bahia, Universidade Federal da Bahia, como requisito para a obtenção do grau de Doutor em Medicina e Saúde.

Orientador: Prof. Dr. Luís Fernando Fernandes Adan

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Salvador

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DEDICATÓRIA

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SUMÁRIO

LISTA DE QUADROS, FIGURAS E TABELAS.

LISTAS DE ABREVIATURAS E SIGLAS

AD	Acurácia diagnóstica
ATC	Anaplastic Thyroid Carcinoma
BRAF	Proto-Oncogene B-Raf
CA19-9	Carbohydrate Antigen 19-9
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CK - 19	Citoqueratina 19
CXCR4	CXC chemokine receptor 4
DAP IV	Dipeptidyl Aminopeptidase IV
E	Especificidade
DPO	Dual-Priming Oligonucleotide
ELISA	Enzyme-Linked Immunoabsorbent Assay
F-	Falso Negativo
F+	Falso Positivo
FISH	Fluorescence in situ hybridization
FRA-1	Fos-related antigen 1
FTC	Follicular Thyroid Carcinoma
FNA	Fine Needle Aspiration
FP	False Positives
FN	False Negatives
FVPTC	Follicular Variant Papillary Thyroid Carcinoma
GAL-3	Galectina 3
GLUT1	Glucose transporter-1
HCC	Hurthle Cell Carcinoma
HNK1(CD57)	Human Natural Killer 1
HMGI	High Mobility Group I
hTERT	Human Telomerase Reverse Transcriptase
HMFG2	Human Milk Fat Globule
LILACS	Literatura Latino-Americana e do Caribe em Ciências da Saúde
MASA	Mutant Allele-Specific Amplification

MTC	Medullary Thyroid Carcinoma
MET	Mesenchymal epithelial transition factor
PAAF	Punção Aspirativa por Agulha Fina
PCR	Polymerase chain reaction
PTC	Papillary Thyroid Carcinoma
PPARgamma	Peroxisome proliferator activated receptor gamma
KS	Keratan-sulphate
RAS	Proto-Oncogene Ras
RET	Proto-Oncogene Ret
S	Sensibilidade
Tg	Tireoglobulina
TPO	Tireoperoxidase
TRAP	Telomere Repeat-Amplification Protocol
TP	True Positives
TN	True Negatives
UFBA	Universidade Federal da Bahia
UFCG	Universidade Federal de Campina Grande
VDAC1	Voltage Dependent Anion Chanel 1
VP -	Valor Preditivo Negativo
VP +	Valor Preditivo Positivo

1 RESUMO

USO DE MARCADORES MOLECULARES EM AMOSTRAS OBTIDAS DE PUNÇÃO ASPIRATIVA PRÉ-OPERATÓRIA DE TIREOIDE: ANÁLISE SECUNDÁRIA DE DADOS

A punção aspirativa por agulha fina (PAAF) se constitui no método mais importante para a avaliação das doenças nodulares da tireoide. Contudo, em alguns casos a amostra citológica obtida desse procedimento se revela insuficiente ou apresenta características que dificultam ou impedem a definição do caráter benigno ou maligno da lesão. Ao se estabelecer a dúvida, a conduta subsequente consiste na remoção cirúrgica da tireoide, em grande parte desnecessárias, porquanto exames posteriores revelam a condição benigna do nódulo. Assim, nos últimos anos, tem se buscado encontrar marcadores moleculares que elevem a acurácia diagnóstica da PAAF, particularmente para as lesões indeterminadas. O volume crescente de experimentos publicados sobre um ou diferentes tipos de marcadores passou a justificar a necessidade de se reunir, minimamente, essas informações, como forma de agregar evidências e nortear o desenvolvimento de pesquisas futuras. A partir de argumentos de busca e critérios previamente definidos, 95 artigos foram selecionados nos indexadores PUBMED, MEDLINE, SCOPUS e LILACS. Foram identificados 36 marcadores submetidos à análise em amostras de PAAF pré-operatória de tireoide, mas apenas 10 (Galectina-3, CK-19, HBME-1, TPO, CD44, Telomerase, DAP IV, RAS, RET e BRAF) foram avaliados em mais de dois estudos, seja em painel ou individualmente. Do conjunto de estudos foram obtidos os valores mínimos, máximos e médios da sensibilidade, especificidade, valor preditivo positivo, valor preditivo negativo e acurácia diagnóstica assim como foram identificadas as limitações e vantagens do uso de cada marcador. A mutação B-RAF, pela inquestionável especificidade, e a Galectina-3 pela regularidade de resultados médios, multiplicidade de localizações e multifuncionalidade no âmbito celular, foram percebidos como detentores das evidências mais expressivas no esforço para reduzir a incerteza diagnóstica em PAAF pré-operatória de tireoide.

ABSTRACT

The fine needle aspiration (FNA) constitutes the most important method for the evaluation of nodular thyroid disease. However, in some cases the cytological specimen obtained in this procedure is inadequate or has characteristics hinder or prevent the definition of benign or malignant lesion. By establishing a doubt, the subsequent conduct is the surgical removal of the thyroid, largely unnecessary, because subsequent examinations revealed the benign nodule. Thus, in recent years has sought to find molecular markers that increase the diagnostic accuracy of FNA, particularly for indeterminate lesions. The growing number of published experiments on one or more of the different types of markers has started to justify the need to gather the pieces of information as a way to add evidence and guide the development of future research in the area. From the search arguments and criteria previously defined, 95 articles were selected from the electronic databases PUBMED, MEDLINE, SCOPUS and LILACS. From the 36 markers submitted to analysis and identified in preoperative FNA thyroid samples, only 10 (Galectina-3, CK-19, HBME-1, TPO, CD44, Telomerase, DAP IV, RAS, RET and BRAF) were assessed in more than two investigations, be it either in panel or individually. The minimum, medium and maximum values of sensibility, specificity, positive predictive value, negative predictive value and diagnose accuracy were obtained from the group of investigation, as well as the limitations and advantages of the use of each marker were identified. The BRAF mutation, for its unquestionable specificity, and the GAL3, for its regularity of average results obtained here, found in several locations in the cell as well as out of the cell, suggesting multiple functions of this molecule, were observed as holders of more expressive evidence in the effort of reducing the uncertainty of the diagnose in preoperative FNA of thyroid.

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2 INTRODUÇÃO

O aumento do volume da tireoide muitas vezes acontece sob a forma de nódulos tireoideos, que são as alterações mais frequentes, decorrentes de: manifestações benignas (nódulos coloides, cistos simples ou tireoidites), responsáveis por 80% dos casos das doenças da tireoide; de adenomas foliculares, encontrados em 10 a 15% e de cânceres de tireoide, em torno de cinco por cento.

A Punção Aspirativa por Agulha Fina (PAAF) representa um dos procedimentos diagnósticos de primeira escolha no manejo clínico das doenças nodulares da tireoide, pela simplicidade da técnica e baixo custo. A sua adoção, no protocolo diagnóstico, contribuiu, de forma notável, para selecionar pacientes adequados para a ressecção cirúrgica das lesões (*), porquanto, consegue definir, com segurança, entre 65 e 80% dos diagnósticos.

Ainda assim, são reconhecidas as suas limitações, pois o material obtido pode ser inadequado ou insuficiente como consequência de alguns fatores, entre eles, a pouca experiência do executor da técnica e/ou das características do nódulo. Durante a análise, o material também pode apresentar um caráter indeterminado em face do padrão arquitetural e das características citológicas da lesão que, por vezes, podem conduzir a equívocos, dúvidas ou discordâncias, dado que é um diagnóstico dependente de uma interpretação a qual é, frequentemente, baseada em critérios sutis e subjetivos (**).

As lesões indeterminadas, que não definem a existência de malignidade na lesão, têm representado entre 10 e 20% dos diagnósticos citopatológicos realizados em material obtido de PAAF pré-operatória de tireoide. Como consequência dessa situação pouco esclarecedora, muitos pacientes são encaminhados para a remoção cirúrgica da tireoide, que é o procedimento particularmente indicado na ocorrência de nódulos malignos.

(*) Gasbarri A, Marchetti C, Lervasi G, Bottoni A, Andrea NC, Nicolini A, et al. From the bench to the bedside. Galectin-3 immunodetection for improving the preoperative diagnosis of the follicular thyroid nodules. Biomed Pharmacother *2004;* 58:356-359.

(**) Suster S. Thyroid tumors with a follicular growth pattern: Problems in differential diagnosis. Arch Pathol Lab Med 2007;131:345-345

Contudo, na avaliação histopatológica da peça excisada, tem-se observado que, em geral, mais de dois terços dessas lesões, tidas inicialmente como indeterminadas, revelam-se benignas.

As repercussões psicológicas e sociais para os pacientes submetidos a cirurgias, *a posteriori* consideradas desnecessárias, bem como os elevados custos para o sistema de saúde, requerem estudos e pesquisas que reduzam essa imprecisão diagnóstica pré-operatória, através de meios e métodos que ofereçam segurança na condução das doenças nodulares da tireoide.

Há algum tempo, vários autores vêm sugerindo que a utilização de marcadores moleculares, ou biomarcadores, representa uma das alternativas para reduzir o número de falsos positivos e falsos negativos no diagnóstico de lesões nodulares de tireoide. Muitos grupos de pesquisa tentaram e outros vêm tentando elevar a qualidade diagnóstica dessas lesões, através da avaliação da expressão de um marcador específico ou de um painel de marcadores.

Vários estudos, no entanto, são levados a efeito procurando demonstrar a qualidade de um ou de vários marcadores a partir de amostras de tecidos resultantes de tireoidectomias totais ou parciais, porquanto o volume, a qualidade e a disponibilidade do material são notadamente superiores.

No entanto, o principal dilema se situa na fase pré-operatória e todos os esforços devem ser dispendidos sobre o material disponível, na quantidade e qualidade oferecida pela PAAF.

É crescente o volume de marcadores submetidos à análise. Alguns deles foram avaliados numa única oportunidade e nela não se obtiveram os resultados esperados, outros foram e continuam sendo submetidos a estudos, por se vislumbrar, neles, resultados promissores; e novos estão surgindo.

Este estudo, portanto, pretendeu Identificar os principais marcadores moleculares propostos para a distinção entre lesões malignas e benignas em material resultante de PAAF pré-operatória de tireoide.

3 OBJETIVOS

PRINCIPAL:

Identificar os principais marcadores moleculares propostos para a diferenciação entre lesões malignas e benignas, em material resultante de Punção Aspirativa por Agulha Fina (PAAF) de tireoide.

SECUNDÁRIOS:

I)Verificar os valores da Sensibilidade (S); Especificidade (E); Valor Preditivo Positivo (VP+); Valor Preditivo Negativo (VP-) e Acurácia Diagnóstica (AD) dos marcadores submetidos aos estudos de expressão;

II) Identificar os marcadores moleculares detentores de potencial para uma aproximação da precisão diagnóstica pré-operatória dos nódulos tireoidianos.

4 MATERIAIS E MÉTODOS

4.1 DESENHO DO ESTUDO

Análise secundária de dados, obtidos em estudos primários de expressão de marcadores moleculares em material resultante de punção aspirativa por agulha fina (PAAF), em nódulos de tireoide, realizada no pré-operatório.

4.2 OBJETO DO ESTUDO

Levantamento bibliográfico dos estudos constantes nos seguintes bancos de dados eletrônicos: SCOPUS; PUBMED; MEDLINE; LILACS.

Nos artigos recuperados, também foi realizada a busca ativa das referências bibliográficas neles citados.

4.3 RECUPERAÇÃO DE ARTIGOS:

A recuperação de artigos foi realizada mediante: Solicitação junto ao COMUT; Solicitação em cadastro particular no SCAD/BIREME (Serviço Cooperativo de Acesso a Documentos); Artigos de livre acesso nos bancos de dados eletrônicos.

4.4 CRITÉRIOS DE INCLUSÃO

Os critérios de inclusão dos artigos foram os seguintes:

a) Idiomas: Inglês, francês, italiano, espanhol e português;
b) O estudo tenha objetivado, principalmente ou secundariamente, diferenciar lesão maligna de lesão benigna de tireoide, a partir da avaliação da

expressão do marcador molecular, em material resultante de Punção Aspirativa por Agulha Fina (PAAF);

c) Os marcadores tenham sido submetidos à avaliação em amostras obtidas no pré-operatório;
d) Os resultados tenham expressado diretamente ou possibilitado a obtenção do número de verdadeiros positivos (V+), falsos positivos (F+), verdadeiros negativos (V-) e falsos negativos (F-), para cada anticorpo ou oncogene individualmente, mesmo quando um painel de marcadores tenha estado sob análise;
e) O histopatológico de peça resultante de ressecção cirúrgica tenha sido considerado o padrão-ouro de diagnóstico.

4.5 CRITÉRIOS DE EXCLUSÃO

Os critérios de exclusão dos artigos foram os seguintes:

a) Estudos publicados em japonês, chinês, alemão, coreano ou outros idiomas não previstos nos critérios de inclusão;
b) O estudo tenha objetivado, principalmente ou secundariamente, avaliar a expressão do marcador molecular nas seguintes condições:
   - ✓ Apenas em lesões malignas ou apenas em lesões benignas de tireoide;
   - ✓ Em espécimes de tecido obtidos da ressecção cirúrgica total ou parcial da tireoide;
   - ✓ Em material de Punção Aspirativa por Agulha Fina (PAAF) de tireoide realizada nas fases intra-operatória ou pós-operatória.
c) Apresente claras inconsistências entre resultados obtidos para a S (sensibilidade), E (especificidade), VP+ (valor preditivo positivo), VP- (valor preditivo negativo) e AD (acurácia diagnóstica) e os números expressos de V+ (verdadeiros positivos), V- (verdadeiros negativos), F- (falsos negativos) e F+ (falso positivo);
d) Estudos que envolvam testes diagnósticos com órgãos de animais;
e) Estudos cujo objeto seja relato de caso.

4.6 ESTRATÉGIA PARA IDENTIFICAÇÃO DOS ARTIGOS

Uma vez definidos os critérios de inclusão e exclusão, passou-se à fase de identificação dos estudos originais sobre a avaliação de marcadores moleculares em PAAF pré-operatória de tireoide. Os estudos foram identificados através dos bancos de dados:

- ✓ SCOPUS, acessado gratuitamente por acesso institucional (Universidade Federal de Campina Grande) ao Portal CAPES;
- ✓ PUBMED, acessado gratuitamente através do site http://www.ncbi.nlm.nih.gov/pubmed;
- ✓ MEDLINE e LILACS, acessados gratuitamente através da Biblioteca Virtual em Saúde (BVS/BIREME) http://regional.bvsalud.org/php/index.php.

Em decorrência da utilização bastante recente de marcadores moleculares em material de PAAF de tireoide, não foi utilizado filtro por ano de publicação, para evitar a perda de artigos importantes que registram os primeiros feitos. Assim, considerou-se apenas o mês de junho de 2011, como limite de incorporação do registro neste trabalho.

A escolha da MEDLINE e PUBMED resultou da reconhecida importância dessas bases, particularmente, pela disponibilidade de resumos de artigos e publicações de impacto da literatura biomédica internacional.

A SCOPUS é considerada, atualmente, a maior base de dados de citações e resumos de literatura em pesquisa da Web. Contém mais de 27 milhões de registros, 14.000 publicações indexadas, incluindo 4.600 títulos de ciências da saúde. Abrange publicações americanas, europeias e asiáticas, em inglês e outros idiomas. Apesar de criada em 2004, disponibiliza registros a partir de 1966.

A base LILACS foi selecionada por incluir documentos da América Latina e Caribe, que por vezes não são encontrados nas bases anteriormente referidas.

Foi adotada a argumentação Booleana[1] para a busca nas bases de dados, combinando termos que respondessem adequadamente à revisão proposta.

Quatro argumentos de busca foram instituídos, tendo, cada um deles, três colunas. A primeira e a segunda colunas receberam, nos quatro argumentos os mesmos termos, relacionados ao procedimento (punção aspirativa por agulha fina) e ao órgão de interesse (tireoide), respectivamente. A terceira coluna, no primeiro argumento, recebeu a denominação do marcador, entre os mais de 70 potenciais marcadores que vêm sendo analisados**,** de acordo com Manuel e Sáaez, 2010[2]. O segundo, terceiro e quarto argumentos receberam, na terceira coluna, respectivamente, os termos imunocitoquímica, marcador molecular e teste diagnóstico**.**

Tabela 1: Argumentos de busca

ARGUMENTO DE BUSCA 1		
PRIMEIRA COLUNA	SEGUNDA COLUNA	TERCEIRA COLUNA
FINE-NEEDLE ASPIRATION	THYROID	DENOMINAÇÃO DO MARCADOR
ARGUMENTO DE BUSCA 2		
PRIMEIRA COLUNA	SEGUNDA COLUNA	TERCEIRA COLUNA
FINE-NEEDLE ASPIRATION	THYROID	IMMUNOCYTOCHEMICAL
ARGUMENTO DE BUSCA 3		
PRIMEIRA COLUNA	SEGUNDA COLUNA	TERCEIRA COLUNA
FINE-NEEDLE ASPIRATION	THYROID	MOLECULAR MARKER
ARGUMENTO DE BUSCA 4		
PRIMEIRA COLUNA	SEGUNDA COLUNA	TERCEIRA COLUNA
FINE-NEEDLE ASPIRATION	THYROID	DIAGNOSTIC TEST

Fonte: Dados da pesquisa, 2012.

4.7 COMPOSIÇÃO DA EQUIPE

A equipe foi composta por três pessoas, o doutorando e dois discentes, um de graduação em Medicina e outro de Mestrado em Saúde Pública da Universidade Estadual da Paraíba (UEPB).

[1] A argumentação Booleana recebeu esse nome em homenagem ao matemático George Boole. Consiste no uso de operadores de inclusão (OR), restrição (AND) e exclusão (NOT) entre os termos procurados.

[2] Manuel J, Sáaez G. Diagnostic usefulness of tumor markers in the thyroid cytological samples extracted by fine-needle aspiration biopsy. Endocr Metab Immune Disord Drug Targets. 2010;10:47-56.

4.8 PLANEJAMENTO DO ESTUDO

Os descritores foram utilizados separadamente por dois discentes e pelo doutorando, seguindo as seguintes etapas:

I. Inserção dos argumentos de busca propostos nas bases de dados escolhidas, realizando-se, em seguida, o agrupamento e confronto dos resultados obtidos pelas duas pesquisas e eliminação dos artigos repetidos;
II. Leitura dos títulos e resumos ou do artigo completo, quando existente;
III. Arquivamento da publicação selecionada preliminarmente;
IV. Solicitação de artigo completo, quando necessário, ao Serviço Cooperativo de Acesso a Documentos – SCAD (BVS/BIREME), através de acesso particular do doutorando;
V. Leitura do artigo completo;
VI. Seleção ou não do artigo completo, quando confrontado com os critérios de inclusão e exclusão;
VII. Discussão das discordâncias para a avaliação da inclusão ou exclusão, por consenso;
VII. Registro das informações.

4.9 DADOS DE SISTEMATIZAÇÃO:

Os dados de sistematização, constantes do Anexo B, foram os seguintes:

- Tamanho da amostra;
- Distribuição da amostra por sexo;
- Distribuição etária dos participantes da amostra;
- Aprovação do experimento por Comitê de Ética ou equivalente;
- Número e tipos de lesões benignas consideradas na investigação;
- Número e tipos de lesões malignas consideradas na investigação;
- Tipo de preparação citológica utilizada na investigação;

- Diluição ou concentração do biomarcador (quando aplicável);
- Calibre da agulha utilizada na PAAF;
- Número de citopatologistas envolvidos na análise.

4.10 QUALIDADE DOS ARTIGOS

Os artigos foram classificados segundo parâmetros adaptados de Figueiredo & Tavares-Neto (2001)[3]:

Tabela 2: Classificação de artigos

CONCEITO	PRESENÇA DAS INFORMAÇÕES CONSIDERADAS (%)	PUBLICAÇÃO
A	80% – 100%	EXCELENTE
B	50% – 70%	REGULAR
C	0 – até 40%	FRACA

Fonte: Figueiredo & Tavares-Neto (2001).

4.11 TRATAMENTO DOS DADOS DE SISTEMATIZAÇÃO

Após a coleta e seleção qualitativa dos dados dos artigos, eles foram implantados em planilha eletrônica do *Excel 2007.* A análise foi procedida através de:

a) Análises descritivas daquelas variáveis passíveis de expressão absoluta e percentual.
b) Inserção dos dados relativos à expressão do marcador molecular em uma tabela de contingência 2x2 e dela obtidas as grandezas que representam as propriedades dos testes diagnósticos:

[3] Figueiredo GC, Tavares-Neto J: Estruturação de um banco de dados para análise secundária de informações em relatos ou série de casos. Rev. Bras. de Ortopedia 36:203-211,2001

	HISTOPATOLÓGICO		
REATIVIDADE DO ANTICORPO OU MUTAÇÃO	MALIGNO	BENIGNO	TOTAL
POSITIVA	V+	F+	(V+) + (F+)
NEGATIVA	F-	V-	(V-) + (V-)
	(V+) + (F-)	(F+) + (V-)	N

Notas:

(1) Sensibilidade – expressa a probabilidade de um teste dar positivo na presença da doença, isto é, avalia a capacidade de o teste detectar a doença quando ela, de fato, está presente: SENSIBILIDADE = V+/malignos x 100;

(2) Especificidade – expressa a probabilidade de um teste dar negativo na ausência da doença, isto é, avalia a capacidade de o teste afastar a doença quando ela, de fato, está ausente: ESPECIFICIDADE = V-/benignos x 100;

(3) Valor Preditivo Positivo – expressa a probabilidade de um paciente, com teste positivo, estar doente: VALOR PREDITIVO + = V+/Total reatividade + anticorpo x 100;

(4) Valor Preditivo Negativo – expressa a probabilidade de um paciente, com teste negativo, não estar doente: VALOR PREDITIVO – = V-/Total reatividade – anticorpo x 100;

(5) Acurácia Diagnóstica – é a proporção de acertos de um teste diagnóstico, ou seja, a proporção entre os verdadeiros positivos e negativos em relação a todos os resultados possíveis: ACURÁCIA DIAGNÓSTICA = (V+) + (V-)/N.

5 ARTIGOS

5.1 ARTIGO 1

TÍTULO: Doença nodular da tireoide: dificuldades e perspectivas no diagnóstico pré-operatório – revisão da literatura.

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Informamos que o artigo intitulado: DOENÇA NODULAR NA TIREOIDE: DIFICULDADES E PERSPECTIVAS NO DIAGNÓSTICO PRÉ-OPERATÓRIO (REVISÃO DA LITERATURA), de autoria dos Drs.: Homero Gustavo Correia Rodrigues, Alana Abrantes Nogueira de Pontes e Luis Fernando Adan, foi aprovado pelo Conselho Editorial da Revista Brasileira de Medicina, e será publicado segundo ordem cronológica de aprovação de artigos.

Atenciosamente,

Sônia Lisboa

DOENÇA NODULAR DA TIREOIDE: DIFICULDADES E PERSPECTIVAS NO DIAGNÓSTICO PRÉ-OPERATÓRIO – REVISÃO DA LITERATURA

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RESUMO

Embora seja considerado o método de diagnóstico pré-cirúrgico mais preciso para a identificação de um nódulo maligno de tireoide, a Punção Aspirativa por Agulha Fina(PAAF) não consegue determinar definitivamente a natureza da lesão em um número importante de casos. Isto ocorre principalmente em certos tipos histológicos nos quais as lesões benignas e malignas podem ter sobreposição de características citomorfológicas. Diante desta situação, um dos maiores desafios na pesquisa do câncer da tireoide é o desenvolvimento de testes diagnósticos complementares à PAAF, que possibilite o esclarecimento pré-operatório das lesões indeterminadas. Durante as últimas décadas tem ocorrido um substancial avanço no conhecimento da biologia tumoral assim como na qualificação de métodos e técnicas laboratoriais, que por sua vez, permitem e estimulam pesquisadores na busca de biomarcadores para resolver este dilema diagnóstico. Neste artigo de revisão, portanto, pretendeu-se abordar as limitações da citologia aspirativa no diagnóstico pré-operatório da doença nodular da tireoide e as principais características dos tipos histológicos, realçando as dificuldades diagnósticas e a premência na elevação da acurácia dos exames realizados sobre amostras obtidas por PAAF, particularmente nas lesões citomorfologicamente consideradas indeterminadas.

Palavras-chave: Tireoide; Punção Aspirativa por Agulha Fina; Carcinoma de Tireoide.

A tireoide pode ser acometida por diferentes doenças decorrentes de alterações morfológicas, funcionais ou autoimunes (1). Essas alterações se expressam, muitas vezes, através do aumento do seu volume, que por sua vez, em muitos casos assumem a forma de nódulos.

Os nódulos são áreas de crescimento exagerado, e podem ser de vários tamanhos, desde alguns milímetros até vários centímetros de diâmetro. Os nódulos de tireoide podem ser únicos ou múltiplos, benignos ou malignos, produtores de hormônio ou não. A etiologia da doença nodular de tireoide é multifatorial, resultado da interação da suscetibilidade genética com os fatores ambientais, como ingesta de iodo e o tabagismo (2). Compreende um espectro que vai do pequeno nódulo achado de forma incidental a um grande bócio multinodular intratorácico (3).

Estima-se em 0,1% a incidência anual de nódulos tireoidianos clinicamente detectáveis na população adulta, com prevalência de 4 - 7% nos estudos que empregam palpação, 30 a 50% em séries que utilizam ultrassonografia e 50% em estudos de autopsia (4-6).

A maioria dos nódulos tireoidianos é causada por doenças benignas, como nódulos coloides, cistos e neoplasias foliculares benignas, de modo que menos de 5% dos pacientes são portadores de câncer de tireoide (7,8).

Embora raro, o câncer de tireoide apresenta um bom prognóstico, sendo responsável por baixa porcentagem de mortes – 0,16% para homens e 0,24% para as mulheres (9).

Quadro 1 – Classificação histopatológica dos tumores malignos da tireoide
a) Carcinoma papilífero
b) Carcinoma folicular
c) Carcinoma Medular
d) Carcinoma indiferenciado/anaplásico

Fonte: TNM Classification of Malignant Tumours (10)

Os tumores malignos da tireoide são provenientes de dois grupos celulares, de origens embriológicas distintas. As células C, neuroendócrinas,

produtoras de calcitonina, cujo tumor é o carcinoma medular, e as células foliculares que originam os tumores bem diferenciados (papilíferos e foliculares) e os indiferenciados (11). Pelo menos 94% dos tumores de tireoide correspondem aos carcinomas bem diferenciados (12).

A incidência dos diferentes tipos histológicos pode variar bastante, conforme se leve em consideração os fatores geográficos, ingestão de iodo, indicações cirúrgicas, critérios de avaliação anatomopatológica e outros fatores. No entanto, algumas observações parecem estar estabelecidas na literatura médica, como uma maior incidência das formas mais agressivas, como o carcinoma folicular e anaplásico, em detrimento de formas menos agressivas, como o carcinoma papilífero, nas regiões de bócio endêmico (13).

O carcinoma papilífero de tireoide (CPT) corresponde a 85% dos casos de neoplasia maligna da tireoide (14). A forma do núcleo, incluindo a presença de sulcos nucleares e inclusões, acoplado com as mudanças na distribuição da cromatina, continua a ser o critério microscópico básico para o diagnóstico citológico do CPT(15). Suas principais variantes histopatológicas são: clássica, folicular, de células altas, de células colunares e sólida (esclerosante) (16). Cada uma dessas variantes apresenta fenótipos diferenciados, com alterações nas taxas de morbidade e mortalidade. As de maior agressividade incluem as variantes de células altas, sólida e de células colunares, sendo esta a mais grave, principalmente por invasão de linfonodos linfáticos, rápido crescimento, altas taxas de recorrência local e metástases para pulmão, cérebro e osso (17).

A Organização Mundial de Saúde (OMS) define o carcinoma folicular de tireoide (CFT) como uma neoplasia epitelial maligna com diferenciação para as células foliculares na qual faltam as características nucleares do carcinoma papilífero (10). É mais comum em regiões com dietas insuficientes em iodo e representa cerca de 10 a 20% de todas as neoplasias primárias da tireoide (18). A característica que define o carcinoma folicular e o distingue de outras lesões foliculares, benignas ou malignas, é a invasão capsular e/ou vascular, o que não é possível determinar citologicamente (19). Com base na medida da invasividade, carcinomas foliculares são classificados como tumores extensamente invasivos ou minimamente invasivos. No entanto, há

controvérsias sobre a extensão da invasão capsular e vascular para que um tumor seja considerado minimamente invasivo (20).

O carcinoma medular da tireoide (CMT) compreende entre 5% e 10% dos cânceres da glândula (18). Esta neoplasia tem a calcitonina como marcador tumoral extremamente sensível para o diagnostico e seguimento (21). Histologicamente consiste em lâminas de células fusiformes, redondas ou poligonais, separados por estroma fibroso, formando um padrão característico de tumores endócrinos. Os núcleos são geralmente uniformes com figuras mitóticas raras. O citoplasma é eosinofílico com uma aparência granular. Depósitos amiloides são vistos em 60-80% das células tumorais. Quando os elementos pseudopapilares ou células gigantes estão presentes, o CMT pode ser confundido com carcinoma anaplásico, tumor de células de Hürthle ou carcinoma papilífero de tireoide(22). Ocorre de forma esporádica ou não hereditária em 75 a 90% dos pacientes. Nos demais, é uma doença hereditária autossômica dominante com alto grau de penetrância e variabilidade de expressão podendo fazer parte de três síndromes clínicas distintas dependendo dos órgãos envolvidos: neoplasia endócrina múltipla (NEM) 2A, (NEM) 2B e carcinoma medular familiar (23).

O carcinoma indiferenciado da tireoide, também chamado anaplásico, corresponde a cerca de 3% dos tumores glandulares e é um dos tumores mais agressivos que acomete a espécie humana. Histologicamente apresenta uma desorganização tecidual intensa com variantes escamoide, alterações celulares que lembram carcinomas epidermoides, sarcoma-like e de células gigantes (com núcleos bizarros e múltiplos) que substituem o tecido glandular normal (24).

Um dos aspectos mais importantes da avaliação da doença nodular da tireoide é a exclusão de neoplasia. Os fatores que sugerem o diagnóstico de carcinoma tireoidiano incluem principalmente: a) alta probabilidade: história familiar de carcinoma medular de tireoide ou neoplasia endócrina múltipla (NEM), crescimento tumoral rápido, nódulo muito firme, fixação em estruturas adjacentes, paralisia das pregas vocais, linfadenopatia regional, metástases à distância; e b) moderada probabilidade: idade < 20 ou > 60 anos, gênero masculino, história de irradiação da cabeça ou pescoço, textura firme -

possivelmente fixação, nódulo > 4 cm em diâmetro e parcialmente cístico, sintomas compressivos como disfagia, disfonia, rouquidão, dispneia ou tosse (3).

Em 2007, a Sociedade Brasileira de Endocrinologia e Metabologia (SBEM) elaborou, por consenso, as diretrizes brasileiras no manejo dos nódulos tireoidianos. Neste documento é estabelecido que se a ultrassonografia cervical mostrar nódulo menor do que 1 cm e não houver qualquer fator de suspeita, seja na história e exame físico, seja na ultrassonografia, o paciente poderá ser seguido apenas clinicamente. Nódulos acima de 1 cm ou suspeitos à clinica e/ou ultrassonografia devem ser sempre submetidos a PAAF(25).

A PAAF é considerada o método de diagnóstico pré-cirúrgico mais preciso para a identificação de um nódulo maligno de tireoide (26). Consiste em puncionar o nódulo 4 a 6 vezes, sob orientação ecográfica, utilizando-se agulha 25 x 0,6 mm (23 G) e seringa de 5 a 10 mL, aplicando-se pressão negativa de aproximadamente 1 a 2 mL (27). É um procedimento ambulatorial, relativamente barato e fácil de realizar. As complicações são raras e envolvem principalmente o desconforto local (28).

A citologia aspirativa apresenta, entretanto, algumas limitações, pois é dependente do operador da punção-biópsia, do intérprete da citologia, da dificuldade do diagnóstico diferencial da lesão folicular e dos resultados falso-negativos de muitas séries, que não incluem o exame histológico na avaliação (4).

Os problemas envolvidos na interpretação morfológica de lesões da tireoide com um padrão de crescimento folicular têm sido repetidamente abordados pela literatura. Variabilidade e discordâncias diagnósticas são reveladas em diversos estudos (29 - 34).

O termo folicular é frequentemente usado por patologistas para designar tanto as células do parênquima da tireoide, como para descrever a arquitetura ou padrão de crescimento, ou seja, padrão folicular. No Quadro 2, encontram-se nominadas as lesões de tireoide que se apresentam como proliferações foliculares:

Quadro 2 – Classificação das lesões foliculares
• Nódulos (hiperplásicos e adenomatosos);
• Adenoma
• Carcinoma Folicular: ✓ Minimamente invasivo; ✓ Grosseiramente encapsulado, angioinvasivo; ✓ Amplamente invasivo;
• Carcinoma papilífero, variante folicular;
• Carcinoma medular, variante folicular;
• Tumores híbridos.

Fonte: Baloch & LiVolsi (35)

Os principais problemas parecem envolver a distinção entre nódulos hiperplásicos e adenoma folicular, entre adenoma folicular e carcinoma folicular minimamente invasivo, e entre adenoma folicular e variante folicular do carcinoma papilífero (2,33 - 37), porquanto, entre estas lesões podem ocorrer sobreposições de características citológicas.

Na citologia, os nódulos podem ser classificados como benignos (60–70%), malignos (5–10%), indeterminados (10–20%) ou inadequados (10–15%). Quanto aos nódulos diagnosticados como inadequados na citologia, estes podem ser submetidos a uma nova PAAF. No entanto, os nódulos classificados como indeterminados, ou de padrão folicular, representam um dilema clínico devido à dificuldade de classificá-los como benignos ou malignos (5).

Essa incerteza diagnóstica termina por conduzir pacientes a se submeterem a procedimentos cirúrgicos, e todos os seus riscos, não como um ato terapêutico, mas diagnóstico. O resultado disso é que a maioria é operada e, no exame histopatológico, mais de dois terços são classificados como benignos, tendo o procedimento cirúrgico sido realizado sem necessidade, gerando altos custos hospitalares e causando eventuais morbidades relacionadas com a cirurgia radical da tireoide (8,38).

A despeito disso, Bertelli *et al*.(39) relatam estudo com 69 pacientes, entre 65 e 84 anos de idade, submetidos à tireoidectomias. Todos possuíam o diagnóstico clínico de bócio (17 bócios uninodulares atóxicos, 43 bócios multinodulares atóxicos, 01 bócio difuso atóxico e 08 bócios multinodulares tóxicos). Neste mesmo estudo, os pacientes submetidos à punção aspirativa, a citologia benigna representou 39,1% dos casos e a maligna, 14,5%. Laudos suspeitos de malignidade somaram 40,6% dos casos. A indicação de cirurgia, portanto, para 55% (38 pacientes) dos pacientes foi por nódulo maligno ou suspeito de malignidade. No estudo anatomopatológico das peças resultantes das tireoidectomias, o diagnóstico benigno representou 78,3% e apenas 21,7% de malignidade.

Torres (40) analisando o exame citológico de material resultante de PAAF em 61 pacientes chegou a 30(49%) diagnósticos benignos, 7 (11,4%) malignos, 18(29,5%) “proliferações foliculares” e 6(9,8%) citologias não diagnósticas. Todos os pacientes foram submetidos à tireoidectomia. Entre os que apresentaram o diagnóstico de “proliferação folicular”, 11(61%) se apresentaram benignos e 7 (39%) malignos, ao exame histopatológico.

Até agora, as decisões de tratamento têm sido predominantemente baseadas na avaliação dos dados clínicos e citológicos do nódulo. Para minimizar o número de cirurgias desnecessárias ou sérias consequências no atraso do tratamento, vários grupos têm procurado aumentar a sensibilidade e especificidade dos testes pré-operatórios. Durante as últimas duas décadas, um trabalho considerável tem sido feito para encontrar marcadores moleculares para resolver este dilema diagnóstico (41).

Os marcadores tumorais (ou biomarcadores) são macromoléculas presentes no tumor, no sangue ou em outros líquidos biológicos, cujo aparecimento e ou alterações em suas concentrações estão relacionados com a gênese e o crescimento de células neoplásicas (42). Tais substâncias funcionam como indicadores da presença de câncer, e podem ser produzidas diretamente pelo tumor ou pelo organismo, em resposta à presença do tumor (43).

Esses marcadores, em sua maioria, são proteínas ou pedaços de proteínas, incluindo antígenos de superfície celular, proteínas citoplasmáticas, enzimas e hormônios (44). Os mesmos podem ser úteis no manejo clínico dos pacientes com câncer, auxiliando nos processos de diagnóstico, estadiamento, avaliação de resposta terapêutica, detecção de recidivas e prognóstico (43-45), além de auxiliar no desenvolvimento de novas modalidades de tratamento (46). Podem ser caracterizados ou quantificados por meios bioquímicos, imunocitoquimicos ou imunohistoquímicos nos tecidos ou no sangue, e por testes genéticos para pesquisas de oncogenes, genes supressores de tumores e alterações genéticas (44).

Vários potenciais marcadores têm sido identificados e submetidos a experimentos(5,38) em amostras de tecidos pós-cirúrgicos de tireoide ou em material obtido de PAAF nas fases pré-operatória, intra-operatória ou pós-operatória. Ainda assim, não se percebe a incorporação efetiva de nenhum deles na rotina da prática clínica, particularmente pela variável reprodutibilidade, o que enseja controvertidos resultados.

CONCLUSÃO

Diante do conhecimento da alta prevalência de lesões nodulares de tireoide associada à baixa incidência de câncer, fica estabelecida a necessidade de uma avaliação tão seletiva quanto possível na recomendação para a remoção cirúrgica. O esforço incessante de diversos grupos de pesquisa espalhados por diversos países conduzirá, mais cedo ou mais tarde, ao encontro do marcador, ou, mais provavelmente, do painel de marcadores que reduzirá a incerteza diagnóstica na fase pré-operatória das lesões indeterminadas. Contudo, certas qualificações necessariamente serão exigidas daquele marcador ou marcadores entendidos como mais apropriados. Aqui incorporamos as qualificações propostas por Haugen *et al*(47):

- deve ser capaz de distinguir fielmente as lesões benignas das lesões malignas, particularmente em nódulos que são citologicamente indeterminados.
- deve ser confirmado por diferentes investigadores como uma ferramenta útil no diagnóstico de câncer de tireoide;
- deve ser capaz de ser medido facilmente a partir de produtos de PAAF tanto por imunocitoquímica, um ensaio funcional ou alguma forma exata de RT-PCR(Reverse Transcriptase- Polymerase Chain Reaction);
- será de utilidade adicional se tiver significado prognóstico em pacientes diagnosticados com carcinoma da tireoide, bem como proporcionar a visão da patogênese e opções de tratamento.

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5.2 ARTIGO 2

TÍTULO: Use of molecular markers in samples obtained from preoperative aspiration of thyroid.

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Original

Use of molecular markers in samples obtained from preoperative aspiration of thyroid

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Abstract. Several experiments have been carried out in order to find molecular markers that increase the diagnose accuracy of the Fine-Needle Aspiration (FNA), especially for thyroid lesions of undetermined significance. The growing number of published experiments on one or more of the different types of markers has started to justify the need to gather the pieces of information as a way to add evidence and guide the development of future research in the area. From the search arguments and criteria previously defined, 95 articles were selected from the electronic databases PUBMED, MEDLINE, SCOPUS and LILACS. From the 36 markers submitted to analysis and identified in preoperative FNA thyroid samples, only 10 (GAL3, CK-19, HBME-1, TPO, CD44, Telomerase, DAP IV, RAS, RET and BRAF) were assessed in more than two investigations, be it either in panel or individually. The minimum, medium and maximum values of sensibility, specificity, positive predictive value, negative predictive value and diagnose accuracy were obtained from the group of investigation, as well as the limitations and advantages of the use of each marker were identified. The BRAF mutation, for its unquestionable specificity, and the GAL3, for its regularity of average results obtained here, found in several locations in the cell as well as out of the cell, suggesting multiple functions of this molecule, were observed as holders of more expressive evidence in the effort of reducing the uncertainty of the diagnose in preoperative FNA of thyroid.

Key words: Thyroid, Fine needle aspiration, Molecular marker

FINE-NEEDLE ASPIRATION (FNA) represents one of the first choices of diagnose procedure in the clinical management of nodular thyroid diseases, given both its technical simplicity and low cost [1]. The adoption of FNA in the diagnose protocol has contributed to the selection of adequate patients for the surgical resection of the lesions [2], because the procedure can accurately define from 65% to 80% of the diagnoses. However, its limitations are acknowledged, due to the fact that the material obtained may be considered as either inadequate or scarce because of some factors, among them, the little experience of the technical executor and/or the nodule characteristics. The diagnoses can also be of undetermined significance depending on the architectural pattern and on the cytological features of the lesion, which, sometimes, may lead to misunderstandings, doubts or disagreements, for the fact that it is a diagnose that depends on interpretation frequently based on subjective and subtle criteria [3]. Lesions of undetermined significance, which do not define the existence or absence of malignant lesions, have represented from 10% to 20% of the cytopathological diagnoses in materials obtained in preoperative FNA of thyroid. Because of such a non-conclusive situation, several patients are referred to total or partial surgical removal of the thyroid, which is the procedure particularly suitable for the occurrence of malignant nodules. However, during the histopathological evaluation of the excised piece, it has been observed that, in general, more than two thirds of the lesions initially being of undetermined significance are in fact considered as benign. For some time, several authors have suggested that the use of molecular markers, or biomarkers, represent one of the alternatives to reduce the number of false positives and false negatives in diagnosing nodular thyroid. Several research groups have tried, and others have been trying to raise the quality of diagnose of

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these lesions by assessing the expression for a specific marker or for a panel of markers. Several investigations, however, have been tested in order to show the quality of one or several markers from tissue samples resulting from partial or total thyroidectomy, for the volume, quality and availability of the material are admittedly superior. However, the main dilemma lies on the preoperative phase, and all efforts must be made focusing on the available material as well as on the quantity and quality offered by the FNA. The quantity of markers submitted to analysis has been growing, having some of these markers been assessed in one single chance, but it has not been possible to obtain the expected results yet. Some other markers were and have been submitted to investigation, due to the fact that, through them, promising results have been foreseen, and new results have come up. This review of literature is a result of the perception of the need for a careful gathering of such investigations together with their results in order to acquire knowledge about the markers or combinations that have a higher number of evidences, as well as of those markers proper to use and methodologically feasible in material resulting from FNA.

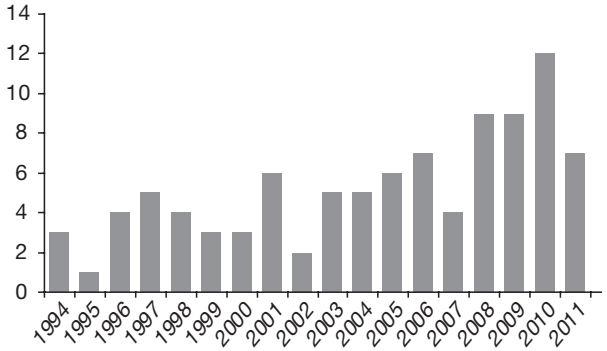

Fig. 1 Distribution of studies per year of publication

Table 1 Systematization elements of primary studies

1. Sample size
2. Sampling distribution per sex
3. Average age of the members of the sample
4. Approval by Ethics Committee or equivalent
5. Number and types of benign lesions considered in the investigation
6. Number and types of malignant lesions considered in the investigation
7. Type of cytologic preparation used in the investigation
8. Dilution or concentration of biomarker (if applicable)
9. Gauge of the needle used in the Fine-Needle Aspiration (FNA)
10. Number of cytopathologists involved in the analysis

Materials and Methods

This investigation is a systematic review with secondary data analysis of other investigations that have used samples of material obtained from preoperative FNA of thyroid – recovered from electronic database PUBMED, MEDLINE, SCOPUS and LILACS –, and also of the active search for the references of such articles, between 1994 and June, 2011 (Fig 1). The inclusion criteria defined were as follows: a) the language the article was published in: English, French, Italian, Spanish or Portuguese; b) the main or secondary objective of the article: to differentiate malignant and benign thyroid lesion from the assessment of the expression of the molecular marker in material resulting from FNA; c) the markers had been submitted to assessment during the preoperative phase; d) the results expressed directly or led to the number of true positives (TP), false positives (FP), true negatives (TF) and false negatives (FN), for each antibody or individual mutation, even when a panel of markers had been analysed; and e) the histopathology of excised piece had been considered as the gold standard of diagnose.

The articles were assessed and the data that composed a systematization form previously elaborated was obtained. The articles were classified according to the volume of information offered (Table 1), following the criteria: Class A (Excellent): from 80% to 100%; Class B (Regular): from 50 to 70%; Class C (Weak): from 0 to 40%.

The results of the expression of the marker as well as of the histopathology presented in each investigation were inserted in a contingency table 2x2 and, from the table, the main quantities assessing the diagnose tests were obtained: sensibility; specificity; predictive positive value; predictive negative value and diagnose accuracy.

Results

After reading and correlation to the defined criteria, 95 articles from different electronic database were included in this study. Most of the studies (76.8%) were related to classes B and C. Only three articles had the total of elements of systematization available. The studies included were produced by research groups from 23 different countries and taken from 43 different periodicals. Most of the articles (56.8%) were from

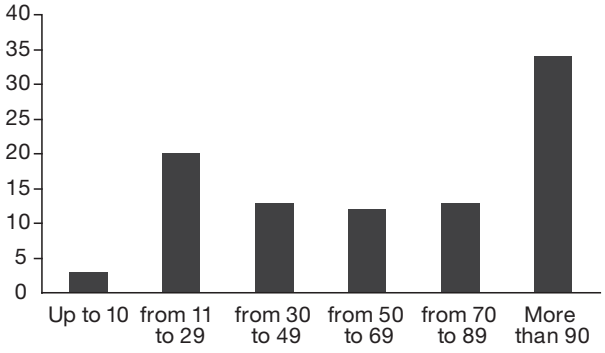

Fig. 2 Number of nodules used in the samples of the studies.

Italy and the United States.

The articles included here have gathered 8,274 thyroid nodules that were submitted to preoperative FNA. The histological subtype of lesion has not been distinguished in eleven investigations, leaving 7,776 identified lesions classified as follows: among the benign lesions there were 2,019 adenomas, 842 goiters, 677 hyperplasias and 204 other lesions; among the malignant lesions there were 3,204 papillary carcinomas and variants, 699 follicular carcinomas, 52 medullary carcinomas and variants; 38 anaplastic carcinomas and 41 other malignant lesions. Among the other 84 investigations in which lesions were distinguished, it was observed that experiments have been carried out on several histological subtypes in 69 of such investigations, being them simultaneously malignant or benign. Regarding the size of the samples, there was a predominance of studies (35.8%) that used samples higher than 90 nodules (Fig. 2). Most of the studies (69.4%) did not differentiate the gender of participants of the sample; 64.2% did not make reference to the participants' age and 57.4% did not indicate the approval of the investigation by any ethics committee or similar.

No reference was made to the dilution of the marker or to the final concentration in 44.5% of the studies, and other 13% followed the manufacturers' orientations. Only 29 (30.5%) studies indicated the participation of more than one cytopathologist in the assessment of results of the expression of the markers. Most of the studies (58.9%) did not make reference to the gauge of the needle used to puncture the nodules.

The articles selected refer to expression studies of 36 different markers in preoperative FNA of thyroid. The most highlighted ones were GAL3 (Galectin-3, a member of the beta-galactosidase binding protein family), BRAF (Proto-oncogene B-RAF), RET (Proto-oncogene RET) and HBME-1(Hector Battifora Mesothelial Antigen-1). These markers were referred to and involved in 81% of the investigations included here.

Thirty (31.6%) out of the ninety-five articles selected included studies with panels of markers (Table 2) and 65 (68.4%) used only one marker. In the studies with markers in panel during use, GAL3, BRAF, RET and HBME-1 have excelled, respectively. In the investigations that used only one marker, there was clear predominance of GAL3 and of the BRAF mutation.

The streptavidin-biotin-peroxidase complex was the predominant method in the immunocytochemical investigations (Table 3), followed by the detection by free biotin. Regarding the mutations or gene expressions, almost all of them were analysed by polymerase chain reaction (PCR) through different techniques of detection, such as, respectively: *Direct sequencing*; *Light Cicler - PCR*; *Mutant Allele-Specific Amplification* (MASA); *Pyrosequencing*, *Dual-Priming Oligonucleotide* (DPO) - *based multiplex* and *colorimetric mutector assay*.

The immunocytochemical studies composed predominantly their samples in the form of smears (56.6%), followed by a cell block (39.5%), immunoblotting (1.9%) and nonspecified (1.9%). Regarding mutations or gene expression, the samples consisted of liquid based preparations (38.6%), reprocessing smear for extraction of nucleic acid (31.8%) and wash out fluid (29.5%).

Discussion

First, it seems important to consider that, although it has been acknowledged that some markers have better qualification, sensibility or specificity, for one or more histological subtypes, due to the genetic alterations in lesion [4], either the differences of the main localization of the marker in cell or the morphological characteristics of the lesion [5], the objective of this investigation was not to assess the qualification of the markers concerning one specific histological subtype, but to identify the information register considered to be relevant (elements of systematization) in experiments with molecular markers regarding preoperative FNA of thyroid, as well as how to consolidate values expressed by them through similar methodologies.

The challenges imposed during a review like this must be acknowledged, for the fact that different research protocols are applied to the same theme, lead-

Table 2 Study distribution according to the types and quantity of markers used in panel

Author	Year	Markers used				
Van Hoeven *et al.* [7]	1998	HBME-1	CA19-9	CD15		
Maruta *et al.*	2004	GAL3	CD44			
Bartolazzi *et al.*	2001	GAL3	CD44			
Gasbarri *et al.* [37]	1999	GAL3	CD44			
Cantara *et al.*	2010	BRAF	RET	RAS	TRK*	PAX8*
Salvatore *et al.*	2004	BRAF	RET			
Moses *et al.*	2010	BRAF	RET	RAS		
Nikiforov *et al.*	2009	BRAF	RET	RAS	PAX8**	
Musholt *et al.*	2010	BRAF	RET			
Sapio *et al.* [32]	2007	GAL3	BRAF			
Sapio *et al.*	2007	BRAF	RET	TRK*		
Pizzolanti *et al.*	2007	BRAF	RET			
Domingues *et al.*	2004	BRAF	RET			
Ohori *et al.*	2010	BRAF	RET	RAS	PAX8**	
Raggio *et al.*	2010	HBME-1	GAL3	CK-19		
Bonzanini *et al.* [14]	2008	CK-19	P63			
Saleh *et al.*	2009	CK-19	HBME-1	GAL3	RET	
Micco *et al.*	2008	HBME-1****	TPO	DAP IV		
Torregrossa *et al.* [8]	2010	HBME-1	GAL3	CXCR4		
Franco *et al.*	2009	HBME-1	GAL3			
Torres-Cabala *et al.* [15]	2006	GAL1	GAL3	S100C	VDAC1	
Saggiorato *et al.* [6]	2005	GAL3	HBME-1	TPO	CK-19	KS
Rossi *et al.*	2005	GAL3	HBME-1	RET		
Pineda *et al.* [27]	2003	GAL3	MUC-1	DAP IV		
Asioli *et al.* [21]	2010	GAL3	HBME-1	EMERIN		
Troncone *et al.* [17]	2009	CYCLIN D1	CYCLIND3			
Pisani *et al.* [11]	2003	Ki67	LAMININ			
Aratake *et al.*	2002	GAL3	DAP IV			
Chandan *et al.* [23]	2006	CD-57	GLUT-1*			
Nar *et al.*	2011	CYCLIN A***	CYCLIN B1***			

* There was no mutation in the samples selected. ** Only one mutation present in the sample. *** In FNA samples, the marker was negative for benign and malignant lesions. **** Postoperative FNA.

ing to variations of methodological quality, making it hard to compare previous investigations. Such methodological differences generate huge discrepancies of results among studies of several markers [6].

Although this investigation did not intend to discuss operative characteristics of laboratory techniques to identify markers, the number of differences among techniques and methods used in the studies included must be highlighted. Differences from gauge needles used for puncturing, moving to the dilution or final concentration of the marker, up to the criteria of measurement of immunostaining are identified. Some examples are:

- It was observed that six out of the seven studies analysing CK-19 (Cytokeratin - 19) in preoperative FNA of thyroid, have identified dilution, and all of them were different among themselves, varying from 1:40 to 1:400. This fact was reproduced in different markers;
- Studies assessing the immunostaining of the same marker, the GAL3, presented more than four criteria of assessment: strong; mild; weak or negative; nuclear and/or cytoplasmic immunostaining; < 50% *versus* > 50%; < 10%, from 11% to 49% and > 50%; < 10% *versus* > 10%;
- What qualifies an investigation is the existence of more than one observer, who must intervene independently. About 60% of the studies do not indicate the number of cytopathologists involved in the assessment of results shown by the marker.

It was observed a scarcity of studies (≤ 2 studies) on 26 markers (Tables 3 and 4): CA19-9 (carbohydrate antigen 19-9) and CD15[7], CXCR4(CXC chemokine receptor 4) [8], onfFN (onconfetal fibronectin) [9], HMGI (High Mobility Group I) [10], Ki67 and Laminin [11], Lactoferrin [12], MET(hepatocyte growth factor receptor) [13], p63 [14], S100/VDAC1(voltage dependent anion channel 1)/Galectin-1 [15], PPARgamma (Peroxisome proliferator activated receptor gamma [16],

Table 3 Distribution of markers used in preoperative FNA of thyroid by immunocytochemistry and the average values of sensibility, specificity, positive predictive value, negative predictive value, diagnose accuracy obtained.

Marker	Number of experiments	Average SN	Average SP	Average PV +	Average PV -	Average AC
GAL3	27	79.20	87.26	84.15	81.00	82.96
HBME-1	10	78.30	85.40	85.50	76.20	79.80
TPO	8	96.60	81.75	68.13	96.25	85.00
CK-19	7	85.40	81.29	83.14	85.70	83.50
CD44	5	87.40	77.00	63.60	91.00	78.00
RET	2	81.00	65.50	69.50	65.50	73.00
CD57	2	95.50	89.50	80.00	96.50	90.00
DAP IV	1	90.00	83.00	90.00	83.00	87.00
CA 19-9	1	62.00	97.00	93.00	82.00	85.00
CXCR4	1	92.00	96.00	95.00	92.00	94.00
KI67	1	100.00	70.00	61.00	100.00	80.00
LAMININ	1	48.00	76.00	96.00	60.00	84.00
LACTOFFERIN	1	100.00	97.00	66.00	100.00	70.00
CD15	1	71.00	95.00	88.00	85.00	86.00
MET	1	54.00	83.00	84.00	52.00	65.00
P63	1	62.00	95.00	95.00	64.00	76.00
S100	1	100.00	75.00	90.00	100.00	92.00
VDAC1	1	100.00	25.00	72.00	100.00	75.00
CYCLIN D3	1	79.00	100.00	100.00	89.00	92.00
CYCLIN D1	1	32.00	100.00	100.00	71.00	75.00
GAL-1	1	33.00	100.00	100.00	40.00	54.00
KS	1	48.00	98.00	97.00	55.00	68.00
HMFG2	1	72.00	50.00	61.00	62.00	62.00
TG	1	63.00	20.00	46.00	33.00	43.00
EMERIN	1	64.00	96.00	94.00	70.00	79.00

SN, sensibility; SP, specificity; PV +, predictive positive value; PV -, predictive negative value; AC, accuracy

Table 4 Distribution of markers used in preoperative FNA of thyroid by other methods for detection of marker and the average values of sensibility, specificity, positive predictive value, negative predictive value, diagnose accuracy obtained.

Marker	Method	Number of experiments	Average SN	Average SP	Average PV +	Average PV -	Average AC
BRAF	Nucleic acids extraction and PCR	26	52.35	97.92	99.85	51.62	70.54
RET		11	18.20	88.73	87.00	59.60	55.30
RAS		5	23.00	97.20	82.20	63.20	65.00
HMGA2		2	75.00	96.00	94.00	83.50	87.50
MUC-1		2	74.50	95.50	91.50	85.50	87.50
GAL3		1	100.00	17.00	44.00	100.00	50.00
FIBRONECTIN		1	81.00	100.00	100.00	63.00	89.00
HMGI		1	100.00	100.00	100.00	100.00	100.00
FRA-1		1	100.00	25.00	57.00	100.00	62.00
TELOMERASE	Nucleic acids extraction and PCR for hTERT gene expression	3	84.00	63.00	73.00	79.00	74.00
	TRAP PCR-ELISA	4	52.30	81.00	77.00	72.00	68.00
DAP IV	Cytoenzymology	2	91.00	78.50	74.00	92.50	83.50
	Nucleic acids extraction and PCR	1	87.00	33.00	46.00	80.00	55.00
PPARgamma	FISH	1	20.00	100.00	100.00	46.00	60.00

SN, sensibility; SP, specificity; PV +, predictive positive value; PV -, predictive negative value; AC, accuracy; PCR, Polymerase chain reaction; ELISA, Enzyme-Linked Immunoabsorbent Assay; hTERT, Human Telomerase Reverse Transcriptase; TRAP, Telomere Repeat Amplification Protocol; FISH, Fluorescence *in situ* hybridization.

Cyclin D3 and Cyclin D1 [17], KS (Keratan-sulphate) [6], FRA-1(Fos-related antigen 1) [18], HMFG2 (human milk fat globule) and Tg (Thyroglobulin) [9], Cyclin A and B1 [20], Emerin [21], CD57 (Human Natural Killer 1) [22, 23], HMGA2 (High Mobility Group A2) [24, 25] and MUC-1 (mucin-1) [26, 27], as well as a time difference (≥ 5 years) of the studies of 17 markers. The lack of continuity of research regarding the markers concerning material obtained from FNA of thyroid seems to be associated with certain factors, such as:

a) Significant results obtained about cancer in different organs, but not presenting relevant results concerning cytological samples of thyroid;
b) Samples used in the studies in relatively non-representative numbers, which make it impossible to come to any conclusion;
c) Difficulties with the application and reproduction of methodology and techniques applied in the assessment of the expression of the marker, especially in studies of retrospective nature, while the preparation of the slide is an extremely important factor for the success of the study;
d) The acknowledgement throughout the years of the fact that it is impossible to have one single marker to reduce the number of undetermined preoperative FNA.

Among the most frequent studies (≥ 4 studies), some findings seem to be necessary for evidence regarding the performance of each marker, such as:

a) The fact that the last record of studies about Telomerase and CD44 were obtained, respectively, in 2006 and in 2004;
b) Among the eight studies that used TPO (Thyroperoxidase), six were carried out more than 5 years ago and, between the most recent two studies, one of them used the marker in panel together with HBME-1 and DAP IV (Dipeptidyl Aminopeptidase IV);
c) Several authors [14, 28, 29] mentioned difficulties of interpretation concerning the distribution and intensity of expression of CK-19;
d) Divergences of interpretation of HBME-1staining pattern are reported in several studies [20, 29-31];
e) RET/PTC is not expressed in follicular carcinoma [32], and shows low prevalence in the follicular variant of the papillary carcinoma [33];
f) Absence or insufficiency of follicular carcinoma cases in the studies that assessed the DAP IV;
g) One of the lowest sensibility obtained was attributed to the proto-oncogene RAS, *i.e.*, a huge number of malignant lesions histopathologically confirmed did not show the presence of this mutation.

GAL3 is one of most frequently investigated molecular markers for the diagnose of the thyroid cancer [34], be it in tissues [35] or in cytological material of FNA, as shown in this study. The studies published up to the present have not offered a definite answer for the use of GAL3 in clinical practice. Methodological matters are mentioned by several authors [36-39] as being responsible for the controversial results published regarding GAL3.

Despite the fact that different and possible methodological flaws have been mentioned by several authors, GAL3 has shown in this study, by immunocytochemistry, an explicit uniformity of average value for the sensibility, specificity, positive predictive value, negative predictive value and diagnose accuracy, *i.e.*, in all of them, GAL3 has obtained value equal or superior to 80%. The result represents much more for the continuity of research with the GAL3 with a standardization of procedures [37] than for an alleged ban of its use in FNA of thyroid [36].

The BRAF mutation has presented extraordinary average values of specificity (97.9%) and positive predictive value (99.9%) resulting from the occurrence of only seven false positive results identified in three investigations [40-42], among the 2800 malignant and benign lesions used in the 26 investigations including BRAF. The different methods used in the detection of marker do not seem to be a disadvantage, because of the fact that they present similar results [43].

Finally, the BRAF mutation, for its unquestionable specificity, and the GAL3, for its regularity of average results obtained here, found in several locations in the cell as well as out of the cell, suggesting multiple functions of this molecule, were observed as holders of more expressive evidence in the effort of reducing the uncertainty of the diagnose in preoperative FNA of thyroid.

Conflict of Interest

The authors have declared no conflict of interest.

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5.3 ARTIGO 3

TÍTULO: Contribution of the BRAF oncogene in the pre-operative phase of thyroid carcinoma

PERÍODICO: ONCOLOGY LETTERS

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By: Correia Rodrigues et al.

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CONTRIBUTION OF THE BRAF ONCOGENE IN THE PRE-OPERATIVE PHASE OF THYROID CARCINOMA

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ABSTRACT

Several experiments have been carried out over the last few years aiming at finding molecular markers that show the diagnose accuracy of the Fine-Needle Aspiration (FNA), especially in thyroid lesions considered indeterminate. Using some search arguments and previously defined criteria, 37 articles reporting experiments with the BRAF mutation in pre-operative FNA of thyroid were selected in the electronic databases PUBMED, MEDLINE, SCOPUS and LILACS in order to gather main evidences regarding the possible contribution of the marker in the management of the thyroid carcinoma. There were no cases of positive BRAF in follicular carcinomas (FTC), Hürthle cell carcinomas (HCC) or medullary carcinomas (MTC). From the 11 cases of anaplastic carcinomas (ATC), three have shown positivity for the BRAF mutation. The number of positive BRAF in benignant lesions was not significant. The average prevalence of BRAF positive in papillary carcinomas (PTC) was of 58,6%, while in follicular variants of papillary carcinoma (FVPTC) it was of 29,6%. For lesions diagnosed with indeterminate or suspicious, the average prevalence obtained of BRAF positive in PTC was of 48,5%. The experiments included have indicated a specificity of almost 100% and a high predominance of the BRAF mutation in PTC, distinguishing the marker in the planning and medical management of the papillary carcinoma of thyroid.

Keywords: Fine Needle Aspiration, BRAF mutation. Papillary Thyroid Carcinoma

Running title: BRAF in the pre-operative phase of thyroid carcinoma – Rodrigues et al.

INTRODUCTION

Thyroid malignant alterations are characterized by clinical and pathological varieties. They are the most frequent malignant alterations of the endocrine system and have had a progressive increase in the number of cases over the last years (1). The annual incidence of thyroid nodules clinically detected in adults is estimated in 0,1%, with prevalence of 4-7% in investigations using palpation, 30 to 50% in series that use ultrasound and 50% in autopsy (2, 3). The Fine-Needle Aspiration (FNA) represents the main pre-operative tool to diagnose thyroid nodules, given its technical simplicity and low cost, having sensibility and specificity corresponding to 70-98% and 55-100%, respectively, been reported (4); however, FNA limitations have been recognized, due to the fact that the material obtained may not be adequate or might not be sufficient, for its volume and quality depend on the technical executor and/or on the characteristics of the nodule. FNA may also be indeterminate in light of the architectural pattern and the cytological characteristics of the lesion that, several times, may cause misunderstandings, doubts or disagreement, for the fact that it is a diagnose that depends on an interpretation that is frequently based on subtle and subjective criteria (5). Indeterminate situations that do not define whether the lesion is malignant have represented from 10 to 20% of the cytopathological diagnoses in material obtained from pre-operative FNA of thyroid. Commonly, such limitations determine that patients be submitted to surgery and to all inherent risks, not as a therapeutic act, but as diagnose. Consequently, most patients are submitted to surgery and, during the histopathological exam of the excised piece, over two thirds of the nodules are classified as benignant, showing that the surgery is unnecessary, creating high hospital costs and causing eventual morbidities related to radical surgery of the thyroid (6,7). Several investigations have reported that tests for the identification of common somatic genetic alterations in thyroid cancer may be useful for diagnose clarification in samples obtained in indeterminate or suspicious FNA. The RAF protein through BRAF isoform has been one of the most investigated mutations for the diagnose of nodular thyroid

lesions, in isolation or combined with other oncogenes (RAS, RET / PTC) in cytological material, presenting encouraging results (8). The most frequent mutation observed in BRAF involves the transversion of thymine for adenine at position T1799A in exon 15, which causes the substitution of the amino acid valina for glutamic acid at position V600E of the protein. The change of the amino acid activates the protein, because it provides for constitutive phosphorylation of the adjacent amino acids, giving them oncogenic capacity (9,10). The objective of this study was to gather the experiments carried out with this oncogene and their results as a way to generate knowledge of their real contribution in material from pre-operative FNA of thyroid.

MATERIALS AND METHODS

A broad review of literature was carried out using the principles of systematic review. The search strategy has included the electronic bibliographical data bases PUBMED, MEDLINE, SCOPUS and LILACS from January, 2004 to June, 2011. The keywords “Thyroid” and “Fine Needle Aspiration” were combined with “BRAF” as well as with “Molecular Marker”**.** The inclusion criteria defined were the following: a) the article should be have written in English, French, Italian, Spanish or Portuguese; b) the main or secondary objective of the study should have been to evaluate the expression of the proto-oncogene BRAF, in isolation or in panel, in material from FNA; c) the marker should have been submitted to evaluation in samples obtained in the pre-operative phase; and d) the histopathology of the piece from surgical resection should have been considered gold standard of diagnose. Two of the authors have examined the articles recovered that met the pre-defined criteria, and the articles have provided the data that composed a form of systematization. The information extracted from the experiments have included the year of publication, the name of the periodical, the country where the research group was from, the approval register of the experiment by an ethics committee, the distribution of the sample per gender and age, the number and the histological types of the malignant lesions used in the investigation, the method of analysis of the BRAF gene, the number of cytopathologists involved in the experiment,

and the identification of other markers when the investigation carried out had made experiments in panel.

RESULTS

The 37 experiments included in this study were published in 21 different periodicals and were carried out by research groups in eight countries: the United States – 12, Korea – 10, Italy – 9, China – 2, Germany – 1, Japan – 1, France – 1 and Portugal – 1. All of the articles were written in English, except for one of them, which was published in French. Most of the investigations (73%) have made reference to the approval of the experiment by an ethics committee or equivalent body of research of the institution the group belonged to. No gender differentiation was made regarding the participants in 51,3% of the studies. The other participants have identified themselves as 1.209 female and 446 male. The participants' age was not mentioned in 45,9% of the articles. Among the articles highlighting the age reference, it was possible to observe that the average age was 46,1 years.

All of the experiments have involved 3.029 thyroid malignant lesions, as follows: 2.732 papillary carcinomas of thyroid (PTC), 183 follicular variants of papillary carcinoma (FVPTC), 79 follicular carcinomas (FTC), 19 medullary carcinomas (MTC), 11 anaplastic carcinomas (ATC) and five Hürthle cell carcinomas (HCC) (**Table I**). The Polymerase chain reaction (PCR) – direct sequencing was the method predominantly employed to analyze the presence of the BRAF gene in the samples selected. Several experiments (67,5%) have not registered the number of cytopathologists involved in the process or in the analysis of results. In ten experiments, the BRAF gene was submitted to analysis in panel with other markers, having the oncogene RET/PTC been highlighted (**Table II)**. Most part of the studies (75,6%) has included samples of indeterminate or suspicious FNA – 1.366 lesions altogether (**Table III**).

There were no cases of positive BRAF in FTC, HCC or MTC. From the 11 ATC cases, three have been positive for the BRAF mutation. The number of positive BRAF in benignant lesions was not significant. The average prevalence of positive BRAF in PTC was of 58,6%, while in FVPTC, it was of 29,6%. For

lesions with indeterminate or suspicious diagnose, the average prevalence of positive BRAF obtained in PTC was of 48,5%.

DISCUSSION

The thyroid nodule is a common condition, but, sometimes, it represents a relevant challenge to differentiate benignant from malignant lesions. FNA presents an excellent diagnose precision in most cases; however, an important percentage of FNA samples are indeterminate, justifying the efforts of several research groups to find molecular markers that improve the diagnose accuracy of FNA of thyroid. The FNAs that indicate thyroid cancer are rarely false-positive (8). In this case, it is possible to conclude that a biomolecular study of the lesion would have little or no importance. Nevertheless, even such situations justify new approaches, due to the fact that the cytomorphological study of the lesion is not sufficient for the risk stratification and/or for the proper establishment of medical management measures of the lesion. In this line of thought, the BRAF mutation has received special attention in the last years.

Among the four types of thyroid carcinoma, the PTC is the most prevalent responding for 80% to 90% of all the malignant neoplasms of thyroid (10,11) and its incidence has been growing rapidly in several areas of the world (12). The samples of the experiments here included have revealed an average prevalence of PTC in the order of 96% (2.732+183/3.029). It is precisely in this type of lesion that there is more frequent occurrence of the BRAF mutation. It has been reported that the mutation in PTC is located between 28,8% and 69%(13). In the group of studies here included the average prevalence of 58,6% ± 20,8(range 15-91) was obtained. The PTC is frequently associated to an excellent prognosis and to the low mortality, but not all patients share such a result (14), mainly because of the inaccurate information concerning aggressiveness and the level of the tumor in the pre-operative phase (11).

Several authors (11, 15-17) pinpoint the existence of controversy regarding the surgical planning of patients whose cytological aspirations were malignant or indeterminate, if it is partial or total thyroidectomy. In the option for lobectomy, in some cases, the nodule excised is malignant in the

histopathological exam, which will inevitably demand a second surgery to complete the thyroidectomy, generating additional costs and increase of the possibility of complications and morbidity.

The analysis of the presence of the BRAF mutation in material obtained from pre-operative FNA is a useful strategy for the reduction of such imprecision and controversies. Its specificity is of 100% (18). In other words, it is not identified in benignant lesions, being present only in malignant lesions, particularly in thyroid papillary carcinoma, not identifying them totally (low sensibility), although its presence offers the certainty of the result true positive. Moreover, it is suggested that people whose nodules present the BRAF mutation are patients likely to be submitted to total thyroidectomy surgery, independently from the citological results (4). Besides this, in PTC, the BRAF mutation is intimately associated to the extra-thyroidean extension, lymph node metastasis and advanced tumor stages (19, 20), which are the main clinic-pathological risk factors, conventionally associated to the increase of recurrence and mortality taxes for thyroid cancer (21). Although there are controversies (13,46-47), conclusions of meta-analytical studies by *Lee et al* (20) have observed the absence of correlation of the marker with the patient's race, age, gender or tumor size. With such qualifications, the detection of the marker in pre-operative FNA makes it possible the celerity regarding the patient's management, also avoiding other less specific diagnose tests, such as the FNA repetition, scintigraphy or freezing intra-operative assessment (22), as well as the orientation concerning the extension of the surgical resection that prevents the performance of a second surgery (18).

However, it is highlighted that the frequency of the BRAF mutation does not occur in a uniform way among the several PTC variants. It is more frequent in the high cell variant, followed by the conventional type and, finally, in the follicular variant (20). In the specific case of FVPTC in this study, the average prevalence of the BRAF mutation of 29,6% was found. This PTC variant deserves special attention, for the fact that the cytological diagnose may be difficult, because of the superposition of morphological characteristics with benignant or non-neoplastic lesions (17). The presence of positivity for the

BRAF mutation, in itself, is not a predictive factor of worst prognostic in FVPTC, as it is largely considered regarding the other PTC variants (22).

A portion of the experiments here included related to the BRAF mutation was carried out in panel form, mainly with other oncogenes (RAS and RET/PTC). Certainly, their objective was to increase the pre-operative FNA diagnose sensibility, for the fact that the BRAF mutation does not occur together with the RAS mutation or the RET-PTC rearrangement, indicating different genetic alterations in the pathogenesis of the papillary carcinoma (9).

In conclusion, given the relationship between the BRAF mutation and the tumor extension and aggressiveness, it is recommended to analyze the possibility of establishing its detection as a habit in order to apply in morphologically suspicious or indeterminate FNA, as well as to consider it in the pre-operative planning of thyroid cancer.

Table I – Studies, analysis method, number of malignant lesions and results of BRAF detection in FNA preoperative

Study	Year	Analysis method	Histological types Number of malignant lesions of the experiment / BRAF +					
			PTC	**FVPTC**	**FTC**	**HCC**	**ATC**	**MTC**
Salvatore et al.(17)	2004	PCR - Direct sequencing/SSCP	47/23	22/3				
Cohen et al. (22)	2004	PCR Direct sequencing and Mutector assay	27/18	27/4	2/0	1/0	1/1	1/0
Hayashida et al.(13)	2004	PCR - RFLP	21/5					
Xing et al.(23)	2004	PCR - Colorimetric Mutation Detection Method	16/7		5/0	1/0		
Domingues et al.(24)	2005	PCR - Direct sequencing	11/3		1/0			1/0
Chung et al.(25)	2006	PCR - Direct sequencing	107/92		3/0		2/1	
Jin et al. (26)	2006	PCR - Direct sequencing, Colorimetric Mutector assay, LightCycler PCR and Allele-Specific - PCR	45/29	13/2				
Rowe et al. (16)	2006	LightCycler PCR	19/3					
Pizzolanti et al.(27)	2007	Real-Time Allele-Specific-PCR	14/10	2/1	1/0			
Sapio, Posca et al. (28)	2007	PCR-MASA	6/4		1/0			1/0
Sapio, Guerra et al.(18)	2007	PCR - MASA	21/10		5/0			
Kim et al.(29)	2008	PCR- Pyrosequencing	73/63	2/0	3/0		1/0	1/0
Zatelli et al.(4)	2009	PCR - Direct sequencing/RFLP	58/41	16/6	7/0		1/1	6/0
Nikiforov et al. (15)	2009	LightCycler PCR/FMCA	38/18		6/0		2/0	2/0
Moon et al. (30)	2009	PCR - Direct sequencing	84/42					
Marchetti et al.(31)	2009	PCR - Direct sequencing	89/59				2/0	
Bentz et al.(32)	2009	LightCycler PCR/FMCA	24/18	16/6				
Kwak et al. (21)	2009	PCR - Direct sequencing	339/213					
Xing et al.(11)	2009	PCR - Colorimetric Mutation Detection Method	149/68	41/5				
Yip et al.(14)	2009	PCR-FMCA	44/31					
Kim, Song et al. (33)	2009	PCR - Pyrosequencing	101/88					
Jo et al.(34)	2009	PCR - Pyrosequencing	40/30					
Hwang et al. (12)	2010	PCR - Direct sequencing and Allele-Specific - PCR	135/106					
Lin et al. (35)	2010	PCR - Direct sequencing	61/21					
Dujardin et al. (36)	2010	PCR - Direct sequencing	10/7					
Girlando et al. (37)	2010	PCR - Direct sequencing	44/34	16/9				
Musholt et al. (38)	2010	PCR - MASA	22/9		4/0		1/0	1/0
Kim, Lee et al.(39)	2010	DPO -based multiplex PCR	263/221		4/0			1/0
Guo et al..(40)	2010	PCR - Direct sequencing	8/4					
Kwak, Kim et al. (41)	2010	DPO-Based Multiplex PCR	107/86	2/1				

Cont. of Table I

Ohori et al. (42)	2010	LightCycler PCR	20/3					
Moses et al. (43)	2010	PCR - Direct sequencing	70/20	19/3	8/0	2/0	1/0	1/0
Cantara et al. (8)	2010	PCR - Direct sequencing	74/33		3/0	1/0		
Kim, Hwang et al.(44)	2011	PCR- Pyrosequencing	169/154		4/0			
Yeo et al. (45)	2011	PCR- Pyrosequencing	175/95	7/4	6/0			4/0
Adeniran et al. (46)	2011	PCR - Direct sequencing/SSCP	60/40					
Pelizzo et al. (47)	2011	PCR - Direct sequencing / MASA	141/98		16/0			

PTC, Papillary Thyroid Carcinoma; FVPTC, Follicular Variant of Papillary Thyroid Carcinoma; FTC, Follicular Thyroid Carcinoma; HCC, Hurthle Cell Carcinoma; ATC, Anaplastic Thyroid Carcinoma; MTC, Medullary Thyroid Carcinoma; PCR, Polymerase chain reaction; MASA, Mutant Allele-Specific Amplification; DPO, Dual-Priming Oligonucleotide; RFLP, Restriction Fragment Length Polymorphism; SSCP, single strand conformational polymorphism; FMCA, Fluorescence Melting Curve Analysis

Table II – Distribution of studies according to the types and quantity of markers used in panel with the BRAF gene.

Study	Year	Markers used				
Cantara *et al*.	2010	BRAF	RET	RAS	TRK*	PAX8 *
Salvatore *et al.*	2004	BRAF	RET			
Moses *et al.*	2010	BRAF	RET	RAS		
Nikiforov *et al.*	2009	BRAF	RET	RAS	PAX8**	
Musholt *et al.*	2010	BRAF	RET			
Sapio *et al.*	2007	GAL-3	BRAF			
Sapio *et al.*	2007	BRAF	RET	TRK*		
Pizzolanti *et al.*	2007	BRAF	RET			
Domingues *et al.*	2004	BRAF	RET			
Ohori *et al.*	2010	BRAF	RET	RAS	PAX8**	

Table III – Distribution of studies according to the number of thyroid papillary carcinomas in the indeterminate or suspicious cytological samples and the number of positive BRAF mutations.

Study	Total of FNA	Indeterminate or suspicious lesions / PTC number	Indeterminate or suspicious lesions / BRAF +
Moses et al.	196	137/33(19 FVPTC)	137/13 (3FVPTC)
Cantara et al.	235	95/53	95/23
Nikiforov et al.	86	52/17	52/7
Sapio et el.	144	94/21	94/10
Rowe et al.	24	19/19	19/3
Salvatore et al.	96	34/15(6 FVPTC)	34/4(1 FVPTC)
Xing et al.	45	25/7	25/2
Cohen et al.	91	55/29 (21 FVPTC)	55/5(2 FVPTC)
Musholt et al.	93	19/4	19/1
Dujardin et al.	25	13/7	13/4
Kim et al.	279	80/70	80/50
Kwak et al.	130	30/20	30/16
Ohori et al.	117	117/20	117/3
Moon et al.	91	91/84	91/42
Marchetti et al.	111	52/33	33/18
Bentz et al.	45	17/17	17/3
Jo et al.	101	24/9	24/7
Pizzolanti et al.	156	19/3 (1 FVPTC)	19/2(1FVPTC)
Sapio et al.	132	37/6	37/4
Chung et al.	137	25/5	25/3
Domingues et al.	24	10/1	10/0
Hayashida et al.	21	1/1	1/1
Yeo et al.	209	63/49 (5 FVPTC)	63/14 (3 FVPTC)
Adeniran et al.	72	34/22	34/10
Pelizzo et al.	270	164/45	164/30
Jin et al.	71	12/(*)	12/1
Girlando et al.	91	20/14	20/10
Kim et. al.	103	27/18(2 FVPTC)	27/13

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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6 CONCLUSÕES

I. Os resultados obtidos indicaram que 36 marcadores moleculares tiveram a expressão avaliada sobre material obtido de PAAF pré-operatória de tireoide;

II. Maiores valores médios obtidos:

a) Sensibilidade: TPO; CD44; CK-19 e GAL-3;
b) Especificidade: BRAF; RAS; RET e GAL-3;
c) Valor Preditivo Positivo: BRAF; RET; HBME-1 e GAL-3;
d) Valor Preditivo Negativo: TPO; CD44; CK-19 e GAL-3;
e) Acurácia Diagnóstica: TPO; CK-19; GAL-3 e HBME-1

III. A mutação B-RAF, pela inquestionável especificidade, e a Galectina-3 pela regularidade de resultados médios, multiplicidade de localizações e multifuncionalidade no âmbito celular, foram percebidos como detentores das evidências mais expressivas no esforço para reduzir a incerteza diagnóstica em PAAF pré-operatória de tireoide.

7 CONSIDERAÇÕES FINAIS

Apesar de ter alcançado os objetivos propostos, convém apontar algumas dificuldades e limitações do presente trabalho, a título de considerações finais.

Duas condições específicas acarretaram certa dificuldade na sua realização. A primeira delas, diretamente relacionada às bases de dados: a indexação inadequada. A segunda, diz respeito à qualidade dos estudos primários, em particular quanto aos seguintes aspectos:

- Omissão de informações relativas à metodologia, nos títulos e resumos;
- Os métodos da pesquisa, as características da população do estudo e os procedimentos foram muitas vezes mal relatados;
- Nem sempre as amostras submetidas ao estudo de expressão dos marcadores moleculares eram provenientes de PAAF indeterminada.

Embora não se acredite em alteração substancial dos resultados, mas cumpre assinalar duas limitações dessa revisão:

- Não inclusão de artigos não publicados, teses e dissertações; e
- Não inclusão de estudos em idiomas diferentes daqueles constantes dos critérios de inclusão.

8 PESPECTIVAS DE ESTUDOS

Os conhecimentos e experiências produzidos a partir deste trabalho representam um ponto de partida para uma série de propostas acadêmicas, objetivando a continuidade das investigações e contribuir para a formação de futuros médicos, particularmente despertando o interesse pela pesquisa acerca da temática aqui discutida. Entre estas propostas, destacam-se:

1) Envolver estudantes de graduação do curso de medicina e médicos residentes das áreas de clínica médica e endocrinologia, da Universidade Federal de Campina Grande, com a temática do câncer de tireoide, particularmente em relação às informações epidemiológicas loco-regionais, o diagnóstico e o tratamento. Pretende-se viabilizar essa proposta através das seguintes ações:

a) Abertura de vagas para discentes do curso de medicina nos programas institucionais de iniciação à pesquisa (PIBIC e PIVIC), em projetos relacionados ao tema, propostos pelo grupo de pesquisa Medicina e Saúde (linhas de pesquisa: Endocrinologia e Saúde; Epidemiologia), do qual o autor é integrante;

b) Atualização da revisão sistemática, através dos mesmos critérios de inclusão, mas ampliando os idiomas e bancos de dados eletrônicos, inclusive aqueles que incluem teses e dissertações (*);

c) Execução do projeto de pesquisa intitulado FREQUÊNCIA DE DIAGNÓSTICOS INDETERMINADOS OU SUSPEITOS EM CITOLOGIAS ASPIRATIVAS DE TIREOIDE NO SERVIÇO DE ENDOCRINOLOGIA DO HOSPITAL UNIVERSITÁRIO ALCIDES CARNEIRO (*);

d) Reuniões científicas, participação e apresentação de trabalhos em congressos e encontros especializados;

(*) projetos selecionados para o PROGRAMA INSTITUCIONAL DE VOLUNTÁRIOS DE INICIAÇÃO CIENTÍFICA PIVIC/CNPQ/UFCG/ 2012 EDITAL 02/12 - CP/PROPEX/UFCG.

2) Apresentação de propostas aos órgãos de fomento à pesquisa (FINEP, FAPESQ), objetivando a aquisição de equipamentos específicos e insumos para a pesquisa institucional do grupo;

3) Proposição, às instâncias administrativas da UFCG, da reestruturação dos Laboratórios de Biologia Molecular e Anatomia Patológica do Hospital Universitário Alcides Carneiro, através da aquisição de novos equipamentos e treinamento de pessoal, para a realização de estudos imunocitoquímicos, extração de ácidos nucleicos e análise genômica.

4) Desenvolvimento de uma programação de treinamentos em técnicas e metodologias laboratoriais, objetivando a iniciação de estudantes e técnicos quanto à execução de técnicas, manipulação e preparação de material biológico obtido por PAAF e reprodução de estudos de expressão de marcadores. Pretende-se, com essa atividade, alcançar a possibilidade, a longo prazo, de execução de estudos originais com marcadores moleculares, de iniciativa do próprio grupo de pesquisa.

8 ANEXOS

ANEXO A

ARTIGOS SISTEMATIZADOS

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ANEXO B

FICHA DE DADOS DE SISTEMATIZAÇÃO

UNIVERSIDADE FEDERAL DA BAHIA
FACULDADE DE MEDICINA DA BAHIA
Fundada em 18 de Fevereiro de 1808
Programa de Pós-graduação em Ciências da Saúde

FICHA DE DADOS N°_____

I – IDENTIFICAÇÃO
TITULO DO ESTUDO:
PERÍODICO:
ANO:
AUTOR PRINCIPAL:
IDIOMA:
PAIS DE ORIGEM DO GRUPO DE PESQUISA:
II – DADOS DE SISTEMATIZAÇÃO
a) TAMANHO DA AMOSTRA:
b) DISTRIBUIÇÃO DA AMOSTRA POR GÊNERO: 1 – MASCULINO: _____ 2 – FEMININO:_____
d) APROVAÇÃO EM COMITÊ DE ÉTICA OU EQUIVALENTE 0 – NÃO REFERE 1 – SIM
e) NÚMERO DE LESÕES MALIGNAS: 0 NÃO DIFERENCIA: 1 CA PAPILIFERO E VARIANTES: 2 CA FOLICULAR: 3 CA MEDULAR E VARIANTE: 4 CA ANAPLÁSICO: 5 OUTRAS:
f) NÚMERO DE LESÕES BENIGNAS 0 NÃO DIFERENCIA: 1 ADENOMA FOLICULAR: 2 BÓCIOS: 3 HIPERPLASIAS: 4 OUTRAS:
g) AVALIAÇÃO REALIZADA SOBRE AMOSTRAS EM: 0 – NÃO REFERE 1 – ESFREGAÇO 2 – BLOCO DE CÉLULAS 3 – OUTROS:_____
i) DILUIÇÃO UTILIZADA (quando for o caso): 0 – NÃO REFERE 1 – SIM:_____
j) NÚMERO DE PATOLOGISTAS ENVOLVIDOS NA ANÁLISE: 0 – NÃO REFERE 1 – APENAS UM 2 – MAIS DE UM:_____
k) RESULTADOS OBTIDOS: V + F + V - F -