DENISE DA ROCHA PITTA LIMA DE MORAES

CONCENTRAÇÃO DOS MARCADORES SÉRICOS E PRESENÇA DE SINTOMAS ESPECÍFICOS EM MULHERES COM OU SEM MASSAS ANEXIAIS

CONCENTRATION OF SERUM MARKERS AND PRESENCE OF SPECIFIC SYMPTOMS IN WOMEN WITH OR WITHOUT ADNEXAL MASSES

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CONCENTRATION OF SERUM MARKERS AND PRESENCE OF SPECIFIC SYMPTOMS IN WOMEN WITH OR WITHOUT ADNEXAL MASSES

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Tocoginecologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas para obtenção do título de Doutora em Ciências da Saúde, na área de concentração em Oncologia Ginecológica e Mamária.

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Dedico este trabalho...

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Sumário

Símbolos, Siglas e Abreviaturas

AUC – *Area Under Curve*/ Área sob a curva

BM – *Body mass index*/ Índice de massa corpórea

CA 125 – *Carcinogen antigen*

EIA – Enzima imunoensaio

EOC – *Epithelial Ovary Cancer*

EUA – Estados Unidos da América

FCM – Faculdade de Ciências Médicas

FDA – *Food and Drugs Administration*

FIGO – Federação Internacional de Ginecologia e Obstetricia

HE4 – *Human Epididyms Protein 4*

CAISM – Hospital da Mulher Prof.Dr. José Aristodemo Pinotti - Centro de Atenção Integral à Saúde da Mulher

INCA – Instituto Nacional do Câncer

IP – Indice preditivo

ln – log neperiano

Mean – Média

µl – Microlitro (s)

MUC 16 – mucina 16

nmol/L – Nanomol por litro

OCEDS – *Ovarian Cancer Early Detection Study*

p – *Significance*

pmol/L – Picomol por litro

ROC – *Receiver Operating Characteristic*

ROMA – *Risk for Ovarian Malignancy Algorithm*

TCLE – Termo de Consentimento Livre e Esclarecido

U/ml – Unidades por mililitro

UNICAMP – Universidade Estadual de Campinas

VPN – Valor preditivo negativo

VPP – Valor preditivo positivo

WHO – *Organizaçao Mundial da Saude (World Health Organization)*

xg – Força gravitacional

Resumo

Objetivo: Avaliar a acurácia da mesotelina, CA125, HE4 e índice ROMA na diferenciação de mulheres brasileiras com tumores malignos de ovário daquelas com tumores benignos e ou mulheres saudáveis, e avaliar se os sintomas específicos relatados pelas mulheres podem ser usados em associação à expressão desses marcadores séricos, na diferenciação pré-operatória de neoplasia maligna de ovário. **Sujeitos e Métodos**: Neste estudo de corte transversal foram incluídas 199 mulheres com massa anexial (67 com tumores malignos e 132 com tumores benignos) e 150 mulheres saudáveis. Todas as mulheres com massa anexial, atendidas no hospital do Departamento de Obstetrícia e Ginecologia da Faculdade de Medicina da UNICAMP, foram convidadas a participar do estudo. Um grupo-controle, de mulheres saudáveis atendidas nos ambulatórios de menopausa e planejamento familiar no mesmo hospital, foi selecionado. Após uma explicação sobre os métodos e objetivo da pesquisa, todas as mulheres responderam o questionário com relação aos sintomas específicos. Foram coletados dados sobre a idade e índice de massa corpórea e sangue periférico para quantificação da mesotelina, o CA125 e a HE4. Foi usado o algoritmo de particionamento recursivo baseado no modelo de regressão linear

para verificar a contribuição da idade e de cada marcador sérico no diagnóstico de tumores malignos. Foram comparadas as áreas sob as curvas (AUCs) obtidas através das curvas ROC (Receiver Operator Characteristics) de cada marcador sérico e índice ROMA, para diferenciar mulheres com tumores malignos. Foi calculada a proporção de mulheres com cada um dos 22 sintomas específicos nos grupos com tumores malignos de ovário, tumores benignos e mulheres saudáveis. O sintoma foi considerado positivo quando ocorria mais que 12 vezes ao mês e por até um ano. A proporção de sintomas foi comparada utilizando teste de qui-quadrado ou teste exato de Fischer, quando apropriado. Os 16 sintomas específicos aplicáveis a toda a coorte e para o qual a periodicidade foi verificada foram submetidos à análise pelo Método de Ward para agrupamento hierárquico. Os agrupamentos de sintomas e sintomas isolados identificados foram: abdômen (abdômen inchado e/ou aumento do volume abdominal); dor (dor pélvica, costas e/ou abdominal); pernas inchadas; digestão (estômago cheio e/ou náusea /vômito); alimentação (dificuldade para comer e/ou empachada); sente alguma massa abdominal; diversos (fadiga e/ou dificuldade para respirar); bexiga (urgência em urinar e/ou urinar frequentemente). Foi avaliada a proporção de mulheres com cada agrupamento de sintomas ou sintomas isolados em mulheres com tumores malignos, tumores benignos e saudáveis, através do teste qui-quadrado para tendências. Utilizou-se um algoritmo de particionamento recursivo para verificar a contribuição da idade da mulher, de cada agrupamento de sintomas ou sintomas isolados, estado menopausal, perda de peso e marcadores séricos no diagnóstico de tumores malignos. **Resultados**: O CA125 foi o marcador sérico com maior capacidade para discriminar mulheres com tumores malignos ($p<0,001$).

Entre as mulheres com tumores benignos e CA125 positivo, a HE4 foi positiva em apenas um caso e a mesotelina foi positiva em outro. Em mulheres com CA125 negativo, a idade, a mesotelina e a HE4 não contribuíram para a diferenciação entre mulheres com tumores malignos, tumores benignos e saudáveis. Em contrapartida, em mulheres com CA125 positivo, a HE4 contribuiu significantemente para detecção de mulheres com tumores malignos ($p<0,01$). A AUC da mesotelina foi menor que das AUC dos outros marcadores. O ROMA e o CA125 apresentarm melhores AUCs do que o HE4. A proporção de mulheres com cada um dos agrupamentos de sintomas ou sintomas isolados foi significativamente maior em mulheres com tumores malignos, quando comparadas àquelas com tumores benignos e, destas, comparadas com as mulheres saudáveis (p tendência em todas as comparações <0,01). Após a análise multivarida, as associações mais significativas para detecção de tumores malignos de ovário foram as do agrupamento abdômen ($p<0,001$), expressão do CA125 ($p<0,001$), agrupamento dor ($p=0,01$) e perda de peso ($p=0,03$). **Conclusões:** Em mulheres com CA125 negativo, a mesotelina e HE4 não contribuíram para detecção do carcinoma de ovário. Entretanto, em mulheres com CA125 positivo, a HE4 contribuiu para diferenciar aquelas com tumores malignos. Em mulheres com tumores malignos de ovário, os sintomas específicos, abdômen e dor foram significantemente mais frequentes. Podem ser utilizados em associação ao CA125 na diferenciação de tumores malignos em mulheres com massa anexial.

Palavras-chave: neoplasia ovariana, marcadores biológicos, sintomas.

Summary

Objective: To evaluate the accuracy of mesothelin, CA125, HE4 and ROMA index in the differentiation of Brazilian women with ovarian malignant tumors from those with benign tumors or healthy women; and to evaluate whether the prevalence of specific self-reported symptoms can be used in association to the expression of serum markers for the preoperative differentiation of ovarian malignant tumors. **Study Design**: For this cross sectional study, 199 women with adnexal mass (67 with malignant tumors and 132 with benign tumors) and 150 healthy women were included. All women with adnexal masses, attending the hospital of the Department of Obstetrics and Gynecology of the Unicamp School of Medicine were invited to participate in the study. A control group of healthy women attending menopause and family planning clinics at the same hospital were selected. After an explanation about the study research methods and purpose all women answered a survey regarding specific symptoms. There were also collected data on age and body mass index. Peripheral blood was collected for serum measurements of mesotelina, CA125 and HE4. A recursive partitioning algorithm, based on a linear regression model was used to confirm the contribution of age and each of the serum markers to the diagnosis of

malignant tumors. Comparison of Area Under the Curve (AUC) obtained through Receiver Operator Characteristics (ROC) curves for each of the serum markers and ROMA index were used to differentiating women with malignant tumors. We next calculated the proportion of women with each of the 22 specific symptoms in the groups of women with ovarian malignant tumors, benign tumors and healthy women. We considered a symptom positive if it occurred more than 12 times per month and for less than one year. The proportions were pairwise compared using chi-square or the Fisher exact test where appropriate. The 16 specific symptoms which applied to the entire cohort and for which the periodicity had been ascertained were further subjected to the Ward's Hierarchical Clustering Method. Clusters of symptoms and isolated symptoms were: abdomen (abdominal bloating and/or increased abdomen size); pain (pelvic, back and/or abdominal pain); leg swelling; digestion (indigestion and/or nauseas /vomiting); eating (unable to eat normally and/or feeling full quickly); able to feel abdominal mass; miscellaneous (fatigue and/or difficulty breathing); bladder (urinary urgency and/or frequent urination). We evaluated the trend in proportion of women with each cluster of symptoms in the groups of women with malignant tumors, benign tumors and healthy women using the chi-squared test for trend in proportions. Another recursive partitioning algorithm was used to confirm the contribution of patient age, clusters of symptoms, menopausal status, weight loss and the serum markers to the diagnosis of malignant tumors **Results**: CA125 was the serum marker that had the greatest capacity to discriminate women with malignant tumors ($p<0.001$). Among the women with benign tumors and positive CA125, HE4 was positive in only one case and mesothelin in another case. In women with negative CA125 neither

age nor mesothelin nor HE4 contributed any further to the differentiation between women with malignant, tumors benign tumors and healthy women. In contrast, for women with positive CA125, HE4 contributed significantly to the detection of women with malignant tumors ($p<0.01$). The AUC for mesothelin was smaller than that for all the other curves, and ROMA and CA125 had better AUC than HE4. The proportion of women with each of the clusters of symptoms and isolated symptoms decreased significantly from the group of women with malignant tumors to that with benign tumors and from this group to the healthy women (p for trends in all comparisons= <0.01). After a multivariate analysis the association that contributed the most to the detection of malignant ovarian tumors was that of the abdomen cluster ($p<0.001$), CA125 expression ($p<0.001$), pain cluster ($p=0.01$) and weight loss ($p=0.03$). **Conclusion:** In women with negative CA125 neither mesothelin nor HE4 contributed to detect ovarian carcinoma. HE4 was helpful to differentiate malignant tumors when CA125 is positive. Specific symptoms, abdomen and pain were significantly higher in women with malignant ovarian tumors and may be used along with the CA125 to select women with ovarian malignancy among those with adnexal masses.

Key words: ovarian neoplasms, biomarkers, symptoms

1. Introdução

Anualmente, cerca de 255.000 casos novos de câncer de ovário são diagnosticados no mundo, o que o coloca na sétima posição entre os cânceres mais detectados nas mulheres. Sua letalidade, no entanto, é proporcionalmente a mais alta dentre os cânceres ginecológicos, causando cerca de 140.000 mortes ao ano (1). A prevalência mundial do câncer de ovário é de aproximadamente meio milhão de mulheres em um período de cinco anos (2). A incidência do câncer de ovário é mais elevada nos países industrializados, embora nos países em desenvolvimento esteja concentrado o maior número de casos (96.700 vs 107.500). Na América Latina, a incidência de 8/100.000 mulheres aproxima-se daquela dos países desenvolvidos, que é de 10/100.000 mulheres. Nos países em desenvolvimento como um todo, no entanto, a incidência é mais baixa: 5/100.000 (2, 3). No Brasil, a incidência supera a dos países industrializados em dois registros de câncer de base populacional: Porto Alegre (13/100.000 mulheres) e São Paulo (11/100.000). Na região de Campinas-SP, o câncer de ovário constitui a sétima causa de câncer em mulheres, com uma incidência estimada, no período entre 1991 e 1995, de 5,98 mulheres a cada 100.000. O pico de incidência nesta comunidade situa-se na faixa etária entre 75 e 79 anos (4).

Devido à posição anatômica do ovário, solto e profundo na pelve, supunha-se que o aumento de seu volume não causaria sintomas em fases iniciais, ou seja,

antes de ser muito grande ou que houvesse focos de disseminação. Entretanto, este conceito de “matador silencioso” tem sido contestado e, ao que parece, deveria ser descartado (5). Estudos demonstraram que mulheres com câncer de ovário, em qualquer estádio, apresentam sintomas até mesmo 36 meses antes do diagnóstico (6, 7). Estes sintomas são geralmente subestimados pelas mulheres e por seus médicos, por serem inespecíficos e presentes em muitas outras condições benignas e frequentes. Entretanto, ao examinar-se uma paciente com mais de 50 anos de idade, especialmente na pós-menopausa, que se queixe repetidamente de constante distensão abdominal, mudanças em hábitos intestinais ou urinários, dor abdominal ou pélvica, ou aumento da circunferência abdominal – para a qual seja afastada condição aguda, como gastroenterites etc – deve-se considerar o câncer de ovário entre os diagnósticos diferenciais. É importante, entretanto, entender que esses sintomas são vagos e ainda não totalmente compreendidos. Alguns grupos de pesquisadores estão atualmente conduzindo estudos que visam a estabelecer quais seriam os sintomas específicos de câncer ovariano inicial e a melhor estratégia para diminuir atrasos no diagnóstico. Até que se demonstre o contrário, a triagem de pacientes sintomáticas parece ser a maneira mais promissora de se detectar o câncer de ovário enquanto a doença apresenta um volume menor e de mais fácil controle terapêutico (5,8, 9).

Embora o rastreamento para câncer de ovário não seja recomendado a mulheres da população em geral, muitos estudos estão avaliando a utilização de biomarcadores e ultrassom transvaginal na detecção de tumores iniciais (7, 10, 11). Contudo, após o exame positivo, muitas mulheres são submetidas à

intervenção cirúrgica na forma de laparoscopia ou laparotomia para um diagnóstico preciso, sendo que hoje cerca de uma em cada 10 mulheres é submetida à cirurgia devido a um tumor anexial durante sua vida (12). Nestas, em cerca de 17% dos casos encontra-se um tumor anexial maligno (13).

Por outro lado, aproximadamente 20% das mulheres assintomáticas, em algum momento da vida, poderão apresentar uma massa anexial, geralmente um cisto ovariano, ainda que somente uma pequena porcentagem dessas massas represente uma malignidade ovariana (14). Várias condições benignas e malignas podem estar associadas à massa anexial: tumores primário de ovário – benignos, borderlines e malignos; tumores malignos metastáticos; massas originárias da trompa de Falópio, do útero, do trato gastrointestinal, do trato urinário; de desenvolvimento embriológico remanescente; endometriose; doença pélvica inflamatória e cistos originários de função ovariana normal. Atualmente, o padrão-ouro para diferenciação entre tumores anexiais benignos e malignos é a determinação do tipo histológico no exame microscópico em parafina. Vários cirurgiões têm avaliado as características clínicas do tumor no intraoperatório, em estudo do material por meio de congelação com boa sensibilidade e especificidade (15, 16, 17).

Os tumores anexiais benignos podem ser tratados com segurança por cistectomia, ooforectomia, ou anexectomia sem outras intervenções (13). Entretanto, de acordo com a Federação Internacional de Ginecologia e Obstetrícia (FIGO), se o tumor anexial é maligno, o tratamento e estadiamento da doença devem ser realizados através de cirurgia, que consiste em laparotomia com incisão mediana, compreendendo avaliação cuidadosa de todas as superfícies peritoneais, coleta

de lavados peritoneais ou de ascite (se presente), omentectomia infracólica, linfadenectomia seletiva das cadeias pélvicas e para-aórtica, biópsia ou ressecção de qualquer massa, lesão ou aderência suspeita, biópsias aleatórias das superfícies peritoneais, histerectomia total, salpingooforectomia bilateral e apendicectomia nos tumores mucinosos (18). A cirurgia de estadiamento pode sujeitar as pacientes à grande morbidade cirúrgica e demanda tempo e conhecimento aprimorado. Quando estudado o resultado do tratamento provido por ginecologistas gerais frente àquele realizado por oncologistas ginecológicos mais habituados ao tratamento do câncer de ovário, concluiu-se que, embora as taxas de complicação perioperatórias sejam semelhantes, tanto os resultados do estadiamento quanto da citorredução mostram-se mais adequados quando a cirurgia é realizada por oncologistas ginecológicos, o que pode significar um aumento de até oito meses na sobrevida global (19). Assim, a diferenciação pré-operatória das massas anexiais é fator determinante do prognóstico em mulheres com tumores benignos ou malignos de ovário. Por isso, recentemente, tem-se tentado estabelecer índices de risco de malignidade dos tumores anexiais que facilitem a decisão terapêutica (20, 21, 22).

Frente a uma massa anexial, a diferenciação entre benigna e maligna é realizada essencialmente por ultrassom transvaginal. Embora inicialmente se considerasse que o ultrassom estivesse associado com altas taxas de resultados falsos positivos, quando a técnica é adequadamente padronizada e o exame é realizado por profissionais experientes, sua acurácia na discriminação de tumores anexais está hoje bem definida (21,23, 24). Entretanto, na tentativa de obter melhores níveis de especificidade e sensibilidade, o uso combinado da medida

do CA 125 no soro e o ultrassom transvaginal têm sido usados para mulheres na pós-menopausa (7,25).

O CA125 (MUC16) é um biomarcador tumoral aprovado pelo *Food and Drugs Administration* (FDA) para o monitoramento de recorrência da doença. Nos estudos retrospectivos tem sinalizado a recorrência da doença em até seis meses antes do desenvolvimento dos sintomas. Entretanto, tem sido avaliado extensivamente por seu possível uso na detecção do câncer de ovário em estádio inicial (26). Quando avaliado em mulheres com câncer de ovário, o CA125 está elevado em aproximadamente 50% das mulheres com doença em estádio inicial e em mais de 90% das mulheres com estádios mais avançados (27). Assim, o uso do CA125 como um teste de rastreamento para detecção do câncer restrito ao ovário é limitado pela baixa sensibilidade. Por outro lado, o CA125 está elevado em cerca 1,6% das mulheres na pós-menopausa e saudáveis (28). Assim, embora pareça alta, a especificidade que varia de 96% a 99% não é suficiente (29).

A utilização de um biomarcador tanto para diagnóstico como para rastreamento está baseada na capacidade deste em distinguir doença maligna da doença benigna (30). Portanto, o desempenho do CA125 poderia melhorar quando combinado a um ou mais biomarcadores que aumentassem a sensibilidade sem perderem a especificidade (26,31). Nos últimos anos, a aplicação de técnicas genômicas e proteômicas potencializou a identificação de outros biomarcadores para detecção do câncer de ovário no estádio inicial. Esses biomarcadores incluem antígenos oncofetais, proteínas do tipo mucina, enzimas, coenzimas,

inibidores de enzima, receptores, citocinas, hormônios peptídeos, outras proteínas, fosfolípideos e lipídeos salinizados (32).

Dentre os biomarcadores estudados na detecção do câncer de ovário estão a mesotelina e o HE4, que apresentam boa sensibilidade e especificidade em mulheres com massa anexial, porém isoladamente não são melhores do que quando combinados ao CA125 (14). A comparação da sensibilidade da mesotelina combinada ao CA125, com a sensibilidade do CA125 isoladamente, foi objeto de investigação de três grupos de pesquisadores nos Estados Unidos da América do Norte (EUA). McIntosch et al. (30) e Moore et al. (14) observaram que na combinação desses dois biomarcadores, considerando uma especificidade de 95%, havia o aumento de 13,5% na sensibilidade para detecção de câncer de ovário. No entanto, no estudo de Palmer et al. (26), a combinação dos dois biomarcadores considerando uma especificidade de 98%, não mostrou diferença na sensibilidade.

Quando o HE4 combinou-se com o CA125, no estudo de Moore et al. (14), a sensibilidade e especificidade na detecção de tumores malignos aumentaram significantemente quando comparadas à do CA125 ou do HE4 isoladamente. Em outro estudo realizado por estes autores (33), visando a diferenciar mulheres em grupos de baixo e alto riscos de malignidade, foram incluídas 531 mulheres com massa anexial e a combinação desses biomarcadores possibilitou que 94% das mulheres com diagnóstico de câncer de ovário epitelial fossem classificadas no grupo de alto risco. Já no estudo de Palmer et al. (26), essa combinação, considerando a especificidade de 98%, apresentou sensibilidade de 72% para distinguir mulheres com câncer de ovário das mulheres-controle. Nas com massa

anexial, a combinação CA125 com mesotelina e HE4 apresentou a mesma sensibilidade que a combinação do CA125 e HE4 (14, 26).

Por outro lado, o valor de corte e a especificidade de novos biomarcadores dependem dos seus níveis em mulheres saudáveis. Algumas características dessas mulheres podem influenciar os níveis dos biomarcadores. Lowe et al. (34) avaliaram quais características individuais poderiam influenciar os níveis do CA125, HE4 e mesotelina no estudo que incluiu 155 mulheres na pós-menopausa do Estudo de Detecção Precoce de Câncer de Ovário (OCEDS), sediado em Seattle, EUA. Nesse grupo de mulheres, poucas características pessoais eram significativamente associadas aos níveis do CA125, que estava aumentado em mulheres que usuárias de talco e diminuído nas multíparas. Os níveis HE4 e mesotelina aumentavam com a idade, e o nível de mesotelina diminuía com o aumento do índice de massa corpórea (IMC).

Além disso, considerando-se a grande diversidade do câncer de ovário - inclui mais de 30 subtipos de malignidade, cada um com comportamento histológico, patológico e clinico distinto –, e a baixa incidência da doença, existe um interesse crescente em estudos que avaliam estes biomarcadores e suas combinações, embora no momento nenhum desses marcadores, avaliado isoladamente ou em combinação, tenha atingido o valor preditivo positivo de 10% para detecção do câncer de ovário (12,35). Moore et al. (33) desenvolveram e testaram um algoritmo, hoje conhecido como *risk for ovarian malignancy algorithm* (ROMA), que inclui o CA125 e o HE4 segundo o estado menopausal (33, 36, 37, 38). Este algoritmo tem sido estudado em vários países desde que foi aprovado pelo

FDA em 2011, com resultados variáveis (22, 39, 40). Na metanálise de Li et al. (39), os autores concluem que o ROMA ajuda na diferenciação de carcinomas de ovário quando comparado com tumores epiteliais benignos, mas o HE4 não é melhor que o CA125 isoladamente para carcinoma de ovário ou outros cânceres de ovário. Recentemente, Anton et al. (40), analisando 128 mulheres brasileiras com tumor anexial, concluíram que a HE4 demonstrou a melhor sensibilidade na avaliação de massas anexiais malignas, quando excluíram a endometriose. Também observaram que tanto o CA125, HE4 ou ROMA tiveram baixa sensibilidade na presença de tumores borderlines de ovário.

Atualmente, há uma tendência em tentar analisar conjuntamente os dados da paciente (sintomas), dos marcadores (ROMA) e dos achados ultrassonográficos. Macuks et al. (41) observaram que o HE4 e o CA125, em combinação com a ultrassonografia e o estado menstrual, apresentavam a melhor acurácia quando comparados com qualquer outro desses métodos utilizados separadamente ou em conjunto. Até o momento, não está comprovado o valor real de todos esses índices na avaliação pré-operatória de mulheres com massa anexial. No Hospital da Mulher Prof.Dr. José Aristodemo Pinotti - Centro de Atenção Integral à Saúde da Mulher (CAISM), de 158 mulheres encaminhadas por tumor anexial e CA125 elevado, submetidas à laparotomia entre janeiro de 1996 e março de 1998, 42% apresentavam câncer de ovário. A melhor performance individual foi encontrada com o CA125 (sensibilidade de 78%, especificidade de 75%), seguido pelo escore do ultrassom (sensibilidade de 75%, especifidade de 73%) e estado menopausal (sensibilidade de 73%, especificidade de 69%). A performance obtida com o índice de risco de

malignidade em um ponto de corte de 150 mostrou sensibilidade e especificidade de 79% (42). Mais recentemente, em outro estudo realizado neste hospital, foram avaliados 110 tumores de ovário, sendo 79 (71 ,8%) benignos e 31 (28,2%) malignos. Os critérios de Ultrasom de Timmerman (2008) foram aplicáveis em 91(82,7%) tumores, resultando em sensibilidade de 90%, especificidade de 87%, valor preditivo positivo (VPP) de 69% e valor preditivo negativo (VPN) de 97%. No ponto de corte de 37,4U/mL, o CA125 mostrou sensibilidade de 69% e especificidade de 87,8%, VPP de 69% e VPN de 88%. Quando o CA125 foi associado à idade e aos critérios do ultrassom em um modelo de regressão logística, houve aumento da sensibilidade e da especificidade nos casos ultrassonograficamente malignos (24).

A identificação de marcadores séricos e sintomas específicos poderão contribuir para a diferenciação pré-operatória de neoplasias malignas em mulheres com massa anexial e indicação cirúrgica. Mulheres com neoplasia maligna de ovário devem ser encaminhadas e tratadas em serviços terciários, envolvendo custos financeiros e emocionais elevados. Entretanto, mulheres com tumores benignos podem ser conduzidas no seu local de origem. Por outro lado, sintomas específicos, quando avaliados em relação à sua frequência e duração , poderão ser úteis na identificação de mulheres com câncer de ovário. Todavia, ainda não se sabe se mulheres que apresentam sintomas específicos seriam beneficiadas com a investigação de massas anexiais por dosagem de marcadores séricos ou exames de imagem. Antes de incorporar a avaliação sistemática de sintomas específicos em programas de saúde, é necessário identificar e quantificar esses sintomas em diferentes grupos de mulheres, inclusive as brasileiras.

2. Objetivos

2.1. Objetivo Geral

Avaliar a expressão dos marcadores séricos e a presença de sintomas específicos em mulheres com ou sem massas anexiais.

2.2. Objetivos Específicos

- Avaliar a expressão dos marcadores séricos mesotelina, CA125, HE4 e o índice ROMA em mulheres brasileiras saudáveis e com ou sem massas anexiais, e avaliar a acurácia desses marcadores na detecção de neoplasia maligna de ovário.

- Avaliar a presença de sintomas específicos relatados pelas mulheres, em associação à expressão desses marcadores séricos, na diferenciação de neoplasia maligna de ovário.

3. Publicações

Artigo 1 – **Worthlessness of mesothelin associated to CA125 and HE4 in discriminating ovarian malignancy**

Artigo 2 – **Symptoms, CA125 and HE4 for the preoperative prediction of ovarian malignancy in Brazilian women with ovarian masses**

3.1. Artigo 1

11-02-2012

Dear Biologist denise rocha pitta:

This acknowledges the receipt of your submission entitled, "Worthlessness of mesothelin associated to CA125 and HE4 in discriminating ovarian malignancy," to the American Journal of Obstetrics & Gynecology.

If any items in the submission checklist were omitted, the submission will be considered incomplete and returned to you for resubmission. It is the responsibility of the corresponding author to make sure all authors have been consulted and have approved this submission. We appreciate your attention to these important details.

We will report the results of the manuscript review as soon as possible. Also, you may log onto http://ees.elsevier.com/ajog as an author for details on the processing of your manuscript or to view the new Journal format.

Thank you for your submission to the American Journal of Obstetrics & Gynecology.

Sincerely,

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Worthlessness of mesothelin associated to CA125 and HE4 in discriminating ovarian malignancy

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Condensation of the paper: HE4 is helpful to detect ovarian carcinoma when CA125 is positive but when CA125 is negative neither mesothelin nor HE4 contributed to its diagnosis.

Short title: Mesothelin and ROMA discriminating ovarian cancer in Brazilian women.

Abstract

Objective: evaluate the accuracy of mesothelin, CA125, HE4 and ROMA index in the differentiation of Brazilian women with ovarian malignant tumors from those with benign tumors or healthy women. Study Design: For this cross sectional study, 199 women with adnexal mass (67 with malignant tumors and 132 with benign tumors) and 150 healthy women were included. A recursive partitioning algorithm, based on a linear regression model was used to confirm the contribution of age and each of the serum markers to the diagnosis of malignant tumors. Comparison of Area Under the Curve (AUC) obtained through Receiver Operator Characteristics (ROC) curves for each of the serum markers and ROMA index were used to differentiating women with malignant tumors. **Results**: CA125 was the serum marker that had the greatest capacity to discriminate women with malignant tumors ($p<0.001$). Among the women with benign tumors and positive CA125, HE4 was positive in only one case and mesothelin in another case. In women with negative CA125 neither age nor mesothelin nor HE4 contributed any further to the differentiation between women with malignant tumors. In contrast, for women with positive CA125, HE4 contributed significantly to the detection of women with malignant tumors ($p<0.01$). The AUC for mesothelin was smaller than that for all the other curves, and ROMA and CA125 had better AUC than HE4. **Conclusion:** In women with negative CA125 neither mesothelin nor HE4 contributed to detect ovarian carcinoma. HE4 was helpful to differentiate malignant tumors when CA125 is positive.

Key words: CA125, HE4, mesothelin, ovarian mass, ROMA

Introduction

Ovarian cancer is the deadliest gynecologic cancer, and is recognized as the fifth cause of death due to cancer in women[1]. In Brazil, 2,979 women died as a result of ovarian cancer in 2010. It is estimated that in 2012, 6,190 women will be diagnosed with the disease[2]. Ovarian cancer are diagnosed from a larger group of women presenting with adnexal abnormalities and approximately 10% of all women in the United States will undergo surgery for adnexal mass. Only a small percentage of these women will be diagnosed with an epithelial ovarian cancer (EOC)[3].

Serum analysis of serum markers is a low-cost, non-invasive approach to women with adnexal masses. The techniques are not subject to operator variability, such as imaging analysis[4]. CA125 measurement is an important component in the workup of a woman with an adnexal mass. In women with tumors ultrasonografically classified as malignant, higher CA125 levels were associated with an increased risk of histologically malignant tumor[5]. Unfortunately, in premenopausal woman, abnormal levels of CA125 may be found in common benign conditions such as endometrioma, follicular cysts, cystadenoma, abscess, and pregnancy. High serum concentrations of CA125 are also found in women with pancreatic, stomach, colon and rectum cancers as well as in metastatic disease[6]. This explains the tremendous amount of effort that has been expended to find new ovarian cancer serum markers that could be used together with, or instead of CA125[7].

HE4 (human epididymis protein 4) is a new serum marker for the diagnosis of ovarian cancer. HE4 has been shown to display increased sensitivity for detecting ovarian cancer compared to that of CA125 alone[8]. Moore *et al.*[3] developed a mathematical model to classify patients with a pelvic mass into high-risk or low-risk groups for having EOC, the Risk of Ovarian Malignancy Algorithm (ROMA), which combines CA125 and

HE4 levels along with menopausal status in a logistic regression model. In recent studies, ROMA index has been shown to outperform others Risk of Malignancy Index[9]. In a Brazilian study, CA125, HE4 and ROMA index were used to classify 128 women with ovarian masses: there were no differences in the accuracy of these serum markers for differentiating ovarian cancer[10]. In a meta-analysis including 11 studies from Africa, Europe and North America[11], the ROMA index has been shown to distinguish EOC from benign pelvic masses. The ROMA index was less specific but more sensitive than HE4. However, the ROMA index and HE4 were more specific than CA125. HE4 levels in healthy women were associated with age, so it would be essential to define a specific normal range and cut-off value for premenopausal and postmenopausal women.

Mesothelin is one of the novel serum markers under investigation for the differentiation of adnexal masses[12, 13]. This marker is a membrane-bound protein present in normal mesothelial cells lining the body cavities and is highly expressed in cells from malignant mesothelioma as well as in most EOC[14]. The mesothelin/CA125 interaction may facilitate peritoneal metastasis by initiating cancer cell attachment to the mesothelial epithelium[15]. The Soluble Mesothelin-Related Peptides were found to be elevated in sera of EOC patients. The serum mesothelin and HE4 may be useful for ovarian cancer screening and detection, also they cannot contribute in the diagnosis of early stages of the disease[16, 12, 13].

Although promising, the serum analysis-based algorithms for the differentiation of adnexal masses still lack independent validation[7]. The aim of this study was to compare the concentration of serum markers mesothelin, CA125, HE4 and ROMA index in a sample of Brazilian women with malignant ovarian tumors, benign ovarian tumors and healthy women, and evaluate the accuracy of these markers in the detection of malignancy.

Methods

This cross-sectional study with prospective collection included 199 women with adnexal tumor operated between January 2010 and January 2012, and 150 healthy women attended in that same period. The study was approved by the ethics committee of the Faculty of Medical Sciences/ UNICAMP number 1092/2009 and all women included in the study gave written informed consent. For cases and controls, after the initial interview, we collected information regarding age, menopausal status and measured the body mass index (BMI) (in kg/m^2). Peripheral blood was collected for serum measurements of the mesothelin, CA125 and HE4.

Serum samples and marker assays

All serum samples were stored in aliquots at -80°C until analysis. The level of serum mesothelin was determined using the MESOMARK enzyme immunometric assay Kits (EIA) (Fujirebio Diagnostics, Göteborg, Sweden), according to the manufacturer's instructions. Values were expressed in nanomoles per liter (nmol/L). Automated analysis of CA125 was performed using the OM-MA test (Siemens Medical Solutions Diagnostics, Tarrytown, USA) Values were expressed in units per milliliter (U/mL). The level of serum HE4 was determined using the HE4 enzyme immunometric assay Kits (EIA) (Fujirebio Diagnostics, Göteborg, Sweden) according to the manufacturer's instructions. Values were expressed in picomoles per liter (pmol/L). The Risk of Ovarian Malignancy Algorithm (ROMA) uses the results for HE4 and CA125 to generate a predictive index (PI) for EOC, calculated by the formulas proposed by Moore *at al.* [18] for pre and pos menopause. ROMA index was used to stratify women into high-risk or low-risk groups for having a pelvic mass that is malignant or benign respectively.

Surgery and pathological assessment

Surgery for diagnosis and/or treatment was performed at the hospital of the Department of Obstetrics and Gynecology of Faculty of Medical Sciences (FCM/Unicamp) and the techniques and surgical procedures were chosen and performed according to medical indication. The mean time elapsed between blood collection and surgery ranging from 24h or less for emergency procedures to a maximum of 120 days. The gold standard was the histopathologic diagnosis of surgical specimens, all performed in the Department of Pathologic Anatomy of the FCM/Unicamp following the guidelines of the World Health Organization International Classification of Ovarian Tumors[19]. For statistical purposes, borderline tumors were classified as malignant

Statistical analysis

Data were entered into a Microsoft Excel (Microsoft Corp., Redmond, WA, USA) spreadsheet and analyzed with the R Environment for Statistical Computing Software[20]. All statistical calculations were performed using 95% confidence interval (95%CIs), considering $P < 0.05$ as significant. Women were classified into benign and malignant groups according to tumor histologic diagnosis. The sample size was calculated on the basis of the sensitivity of CA125 and HE4 derived from previous studies, with 5% significance levels, 80% statistical power and 12% error limits for the sensitivity: the minimal number of women with malignant tumors would be 64, and based on the prevalence of malignancy, 122 women with benign tumors would be need for discrimination. The number of controls (healthy women) was estimated at 149, totaling 335 women. We first calculated the proportion of women with positive tumor markers as related to the histological classification of the tumors. Standard cut-off points were used: CA125 was considered positive, when

≥ 35U/ml in pre and post menopaused women. HE4 was considered positive when ≥70pmol/L for pre menopaused women and ≥140pmol/L for post menopaused women[18]. According to manufacturer, for pre menopause the ROMA index was considered high risk when ≥13.1%, and for post menopause when ≥27.7%. Mesothelin was considered positive, when ≥1.5nmol/L in pre and post menopaused women (according to manufacturer).

A recursive partitioning algorithm, based on a linear regression model as described by Hothorn *et al.*[21], was used to confirm the contribution of each of the serum markers and patient age to the differentiation of women with malignant tumors, benign tumors and healthy women. Patient age, mesothelin, CA125, and HE4 serum levels (at the cut-off points mentioned before) were included in the recursive partitioning regression model, and a conditional inference tree was generated. Conditional inference trees estimate a regression relationship by binary recursive partitioning in a conditional inference framework. Branches of the generated inference tree bifurcate when a statistically significant association is detected.

Next, the means and the interquartile ranges of the serum marker concentrations for various groups of women were calculated. We performed pairwise comparisons of the serum marker concentrations in the different groups of women, formed on the basis of the tumor histological classifications and stage (for carcinomas) using the Tukey's Honestly Significant Differences (Tukey's HSD) test. Receiver Operator Characteristics (ROC) curves was generated for each of the serum markers and the ROMA index in differentiating women with malignant tumors from those with benign tumors or healthy women. Finally, pairwise comparisons of the areas under the curves (AUC) were performed using U-Statistics theory and asymptotic normality comparisons as proposed by DeLong *et al.*[22].

Results

Tables 1 and 2, respectively for women with malignant or benign tumors, show the proportions of women with positive serum markers in different histological groups, considering the standard cut-off points. For women with malignant tumors **(Table 1)**, the CA125 levels were positive in 45/67 (67%) women. However, none of the women with mucinous adenocarcinomas and only 3/10 women with borderline tumors had positive CA125 levels. Neither HE4 nor mesothelin levels were found to be positive in women with mucinous adenocarcinomas or borderline tumors. HE4 levels were positive in 28/67 (41.7%) women and mesothelin levels were positive in 17/67 (25.4%) cases. Women with serous, endometrioid, clear cell and mixed carcinomas had the highest proportion of positive CA125, HE4 and/or mesothelin levels. For women with benign tumors **(Table 2)**, CA125 was positive in 23/132 (17%). The highest proportions of women with benign tumors and positive CA125 levels were found among those with cystadenomas, fibromas, teratomas and endometriomas. HE4 levels were found to be positive in only 3/132 (2%) women with benign tumors (two with epithelial tumors and one with endometrioma). Mesothelin was positive in 11/132 (8%) women with benign tumors, evenly distributed along all histological subtypes. Among the 23 women with benign tumors and positive CA125, HE4 was positive in only one case and mesothelin in another case (Data not show in table).

Figure 1 shows the results of the conditional inference tree in which CA125, HE4, mesothelin and women age were included. CA125 was the serum marker that had the greatest capacity to discriminate between malignant tumors, benign tumors and healthy women ($p<0.001$), as shown by the first branch bifurcation of the tree. In women with negative CA125 neither age nor mesothelin nor HE4 contributed any further to the differentiation between malignant tumors, benign tumors and healthy women. In contrast, for

women with positive CA125, the branch bifurcation shows that HE4 contributed significantly to the detection of women with malignant tumors ($p<0.01$).

Table 3 shows that CA125, HE4, ROMA index and mesothelin were statistically higher for women with malignant tumors compared to women with benign tumors and healthy women. Women with benign tumors had serum marker concentrations and ROMA index similar to healthy women. The CA125, HE4, mesothelin concentrations and ROMA index were statistically similar for women with borderline tumors, benign tumors, and healthy women. Women with ovarian carcinoma had CA125, HE4 and mesothelin levels significantly higher than women with malignant stromal tumors, germ cell tumors and healthy women. **Table 4** shows that the CA125, HE4, mesothelin concentrations and the ROMA index were significantly higher in women with stage III/IV carcinomas compared to women with stage I/II carcinomas and healthy women. On the other hand, the serum concentrations of the markers, except for mesothelin, were significantly higher in women with stage I/II carcinomas when compared to healthy women and those with benign tumors**.**

In **Figure 2**, the ROC curves for each marker were compared. The AUC for mesothelin was significantly smaller than that for all the other curves. Although ROMA and CA125 had better AUC than HE4, ROMA didn´t present a better accuracy than CA125 alone.

Discussion

In this sample of Brazilian women, the associated or stand-alone use of HE4 to CA125 was of little, if any, use in the detection of malignant ovarian tumors. The accuracy of CA125 for the detection of malignant ovarian tumors was higher than that of HE4 and mesothelin. Women with an adnexal mass and elevated level of CA125 and HE4 had a significantly higher probability of harboring a malignant ovarian tumor than those with

normal HE4 levels. However, women with mucinous carcinomas and the majority of those with borderline tumors had normal CA125 levels; HE4 and mesothelin were negative, and had no clinical importance. The mean serum levels of the three markers in women with benign tumors were similar to those in healthy women; however the CA125 levels were above the standard cut-off point in a high proportion of the women with cystadenomas, fibromas, teratomas and endometriomas, whereas only two women with benign tumors had positive HE4 and six had positive mesothelin. HE4 can contribute to the identification of women with malign tumors among those with adnexal mass and elevated levels of CA125.

CA125 levels were significantly higher in women with malignant tumors. The same was true for HE4 and mesothelin. Overall, HE4 was negative for all women with malignant tumors who also had negative CA125 levels, resulting in a lower sensitivity and worse accuracy compared to CA125. Consequently, ROMA index was less sensitive than CA125 as a stand-alone marker. In general, only women with carcinomas – primary or metastatic - had elevated levels of mesothelin. This marker was found negative for most women with the other histological types of ovarian tumors. And women with malignant germinative or stromal tumors rarely had positive CA125 and HE4. These data are in accordance with other studies that showed the benefit of HE4 in detecting ovarian carcinoma[17, 23]. Moore *et al.*[3], using the ROMA index classified 94% of the women with carcinomas as at high-risk of having malignant tumors. Considering women with carcinomas, those with serous tumors had higher levels of CA125, HE4 and mesothelin[12]. However, all women with mucinous carcinomas had negative CA125, HE4 and mesothelin measurements regardless of stage. A positive HE4 or mesothelin did not help to determine whose women have mucinous tumors, since women with this histologic type in general have negative CA125 results. Our findings agree with those of Van Gorp *et al.*[7] and Yip *et al.*[24], who studied, respectively,

Belgian and American women. However, Abdel-Azeez *et al.*[12], studying Egyptian women, found that 66% of the patients with mucinous carcinomas who had low levels of CA125 had elevated levels of HE4. In their study, mesothelin was not increased.

In women with carcinomas, the levels of the three markers were significantly higher in women with stage III/IV disease compared to the marker levels in women with stage I/II disease. However, women with stage I/II disease had significantly higher levels of CA125 and HE4 (while mesothelin was at normal levels) compared to women with benign tumors and healthy women. Abdel-Azeez, *et al.*[12] and Fritz-Rdzanek *et al.*[13] found significantly higher levels of mesothelin in women with stage III/IV disease compared to those found in women with stage I/II disease. HE4 levels would be of clinical significance in women with early stage carcinomas, since only half of these women have elevated levels of CA125. Bandiera *et al.*[4] observed that in a group of 21 women with early stage ovarian carcinomas, 11 women had elevated levels of HE4, 15 had elevated levels of CA125 and ROMA index was suggestive of malignancy for only 14 women. Moore *et al.*[18] reported that when ROMA index had a specificity of 75%, its sensitivity was 85.5% for the detection of stage I/II carcinomas in women with adnexal masses. In another study, those authors classified as at high-risk for malignancy 75% of the women with early stage carcinomas[9]. However, in other studies, CA125 is still the best option for the detection of early stage carcinomas, because the HE4 levels have been found to be elevated only in women with advanced stage disease[25].

In our study, women with borderline ovarian tumors rarely had elevated levels of the markers: only 3 out of 10 (30%) women had elevated CA125 levels and none had elevated levels of HE4 or mesothelin. Ponceletet *et al.*[26] reported that 82 out of 202 (40.5%) women with borderline tumors had elevated levels of CA125. Moore *et al.*[3] using ROMA index classified 14 of 19 women with borderline tumors as at high-risk. Since then, in

many studies, CA125, HE4 and ROMA index have been shown to fail in the differentiation of borderline from benign tumors or healthy women[27, 23, 10].

In premenopausal women, HE4 played a better role in predicting which adnexal masses are benign. In these women, several clinical conditions, like endometriomas, are associated with elevated levels of CA125. In these cases, CA125 level is usually elevated. In our series, 8 among the 12 women with endometrioma had a level of CA125 higher than 35U/ml and, HE4 was positive in only one case. Holcomb *et al.*[28] observed that 85% of 229 premenopausal women with adnexal masses actually had benign tumors, and 41% of these women had elevated levels of CA125 and only 8% had elevated levels of HE4. Moore *et al.*[29] found that 37% of 593 pre- or postmenopausal women had elevated levels of CA125 and only 6% had elevated levels of HE4. Because benign adnexal tumors can be treated safely by non-specialized gynecologists, the presurgical differentiation of tumors would benefit medical assistance[30].

In conclusion, our results suggest that women with an adnexal mass and elevated level of CA125, those with elevated level of HE4 had a significantly higher probability of harboring an ovarian carcinoma than those with normal HE4 levels. We also noticed that HE4 was useful in detecting early stage disease. However, in general, women with mucinous carcinomas and borderline tumors had normal CA125 levels and for these women, the determination of serum HE4 levels had no clinical importance. Eventually, HE4 can contribute to the identification of women with benign tumors among those with an adnexal mass and elevated level of CA125, as reported for endometriomas. On the other hand the serum concentrations of mesothelin were seldom increased in malignant tumors. Even worse, mesothelin levels were similar in women with stage I/II carcinomas, women with benign tumors and healthy women. Based on our results, it is not clear enough whether these

new tumor markers should be recommended in the clinical setting for discrimination of Brazilian women with adnexal masses.

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Table 1: Proportions of women with malignant tumors with elevated* serum levels of the tumor markers

MALIGNANT TUMORS	n	(%)	CA125 Positive/Negative	(%)	HE4 Positive/Negative	(%)	Mesothelin Positive/ Negative	(%)
OVARY								
EPITHELIAL								
A)MALIGNANT	37	(55)	29/8	(78)	22/15	(59)	14/23	(38)
Serous adenocarcinoma	18	(27)	16/2	(89)	11/7	(61)	10/8	(55.5)
Endometrioid adenocarcinoma Ŧ	9	(13)	7/2	(78)	7/2	(78)	2/7	(28.5)
Mucinous adenocarcinomaŦ	4	(6)	0/4	(0)	0/4	(0)	0/4	(0)
Clear cell adenocarcinoma	3	(4.5)	3/0	(100)	1/2	(33)	2/1	(66.5)
Mixed adenocarcinoma	2	(3)	2/0	(100)	2/0	(100)	0/2	(0)
Carcinosarcoma	1	(1.5)	1/0	(100)	1/0	(100)	0/1	(0)
B)BORDERLINE TUMORS	10	(15)	3/7	(30)	0/10	(0)	0/10	(0)
Serous	3	(4.5)	1/2	(33)	0/3	(0)	0/3	(0)
Mucinous intestinal pattern	4	(6)	1/3	(25)	0/4	(0)	0/4	(0)
Seromucinous	3	(4.5)	1/2	(33)	0/3	(0)	0/3	(0)
SEX CORD AND STROMAL	8	(12)	5/3	(62.5)	0/8	(0)	0/8	(0)
Granulosa cell tumor	6	(9)	4/2	(66)	0/6	(0)	0/6	(0)
Sertoli-Leydig	1	(1.5)	0/1	(0)	0/1	(0)	0/1	(0)
Ginandroblastoma	1	(1.5)	1/0	(100)	0/1	(0)	0/1	(0)
GERM CELL TUMOR	6	(9)	4/2	(67)	2/4	(33)	0/6	(0)
Immature teratoma	3	(4.5)	3/0	(100)	2/1	(66)	0/3	(0)
Mature teratoma with carcinomatous transformation	1	(1.5)	0/1	(0)	0/1	(0)	0/1	(0)
Dysgerminoma	2	(3)	1/1	(50)	0/2	(0)	0/2	(0)
OVARIAN METASTASIS	4	(6)	2/2	(50)	2/2	(50)	1/3	(25)
From endometrial cancer	2	(3)	1/1	(50)	1/1	(50)	1/1	(50)
From intestinal cancer	1	(1.5)	1/0	(100)	1/0	(100)	0/1	(0)
From unknown primary site	1	(1.5)	0/1	(0)	0/1	(0).	0/1	(0)
TOTAL OVARY	**65**							
EXTRA-OVARIAN	2	(3)	2/0	(100)	2/0	(100)	2/0	(100)
Extra ovarian serous adenocarcinoma	1	(1.5)	1/0	(100)	1/0	(100)	1/0	(100)
Uterine leiomyosarcoma	1	(1.5)	1/0	(100)	1/0	(100)	1/0	(100)
TOTAL	**67**							

*CA125 was considered positive, when ≥ 35U/ml in pre and post menopaused women. HE4 was considered positive when ≥70pmol/L for pre menopaused women and ≥140pmol/L for post menopaused women. Mesothelin was considered positive, when ≥1.5nmol/L in pre and post menopaused women.

Ŧ2 ovarian carcinoma had endometrial carcinoma associated, in these cases considered ovarian carcinoma as the primary tumor

Table 2: Proportions of women with benign tumors with elevated* serum levels of the tumor markers

BENIGN TUMORS	n (%)		CA125 Positive/Negative (%)		HE4 Positive/Negative (%)		Mesothelin Positive/Negative (%)	
OVARY								
EPITHELIAL	40	(30)	5/35	(12.5)	2/38	(5)	4/36	(10)
Serous cystadenoma and/or Serous cystadenofibroma	22	(17)	2/20	(9)	1/21	(4.5)	1/21	(4.5)
Mucinous cystadenoma	10	(8)	1/9	(10)	0/10	(0)	1/9	(10)
Brenner tumor	4	(3)	1/3	(25)	0/4	(0)	0/4	(0)
EPITHELIAL + SEX CORD STROMAL								
Brenner tumor + fibroma	2	(1.5)	1/1	(50)	0/2	(0)	1/1	(50)
Serous cystadenoma + fibroma	1	(0.8)	0/1	(0)	1/0	(100)	1/0	(100)
Serous cystadenofibroma + fibroma	1	(0.8)	0/1	(0)	0/1	(0)	0/1	(0)
SEX CORD AND STROMAL	20	(15)	3/17	(15)	0/20	(0)	1/19	(5)
Fibroma	14	(11)	3/11	(21.5)	0/14	(0)	1/13	(7)
Fibrothecoma	3	(2)	0/3	(0)	0/3	(0)	0/3	(0)
Sclerosing stromal tumor	1	(0.8)	0/1	(0)	0/1	(0)	0/1	(0)
Leiomyoma	2	(1.5)	0/2	(0)	0/2	(0)	0/2	(0)
GERM CELL TUMOR								
Mature teratoma	29	(22)	3/26	(10.5)	0/29	(0)	1/28	(3.5)
NON-NEOPLASTIC OVARIAN	28	(21)	9/19	(32)	1/27	(3.5)	4/24	(14)
Endometrioma	12	(9)	8/4	(66.5)	1/11	(8.5)	1/11	(8.5)
Ovarian edema	1	(0.8)	0/1	(0)	0/1	(0)	0/1	(0)
Functional cysts	11	(8)	0/11	(0)	0/11	(0)	2/9	(18)
Hemorrhagic cysts	2	(1.5)	1/1	(50)	0/2	(0)	0/2	(0)
Ovarian abscess	2	(1.5)	0/2	(0)	0/2	(0)	1/1	(50)
TOTAL OVARY	**117**							
EXTRA-OVARIAN	15	(11)	3/12	(20)	0/15	(0)	1/14	(7)
Hydrosalpinx	3	(2)	0/3	(0)	0/3	(0)	1/2	(33.5)
Adenomyoma or para-uterine leiomyoma	4	(3)	1/3	(25)	0/4	(0)	0/4	(0)
Uterine leiomyoma	3	(2)	1/2	(33.5)	0/3	(0)	0/3	(0)
Morgagni hydatides	2	(1.5)	0/2	(0)	0/2	(0)	0/2	(0)
Tubarian cystadenofibroma	1	(0.8)	0/1	(0)	0/1	(0)	0/1	(0)
Tubarian vascular congestion	1	(0.8)	0/1	(0)	0/1	(0)	0/1	(0)
Tubarian endometrioma	1	(0.8)	1/0	(100)	0/1	(0)	0/1	(0)
TOTAL	**132**							

*CA125 was considered positive, when $\geq$ 35U/ml in pre and post menopaused women. HE4 was considered positive when $\geq$70pmol/L for pre menopaused women and $\geq$140pmol/L for post menopaused women. Mesothelin was considered positive, when $\geq$1.5nmol/L in pre and post menopaused women.

Table 3: Mean serum marker concentrations as related to the histological classification of the tumors

Group	Mean serum concentrations (interquartile range)			
	CA125 (IQR) Unit= U/mL	HE4 (IQR) Unit= pmol/L	ROMA (IQR) Unit= % probability	Mesothelin Unit =nmol/L
All women (199 cases) and 150 healthy women				
Malignant	1207 (795)	275 (276)	42.9 (86.1)	2.5 (1)
Benign	54 (15)	46 (24)	8.1 (7)	0.7 (0.4)
Healthy women	11 (6)	35 (14)	4.7 (4.9)	0.7 (0.3)
Comparisons	p values*			
Malignant vs. benign	<0.01	<0.01	<0.01	<0.01
Malignant vs. healthy women	<0.01	<0.01	<0.01	<0.01
Benign vs. healthy women	0.95	0.89	0.33	0.99
Women with ovarian tumors (172 cases) and 150 healthy women				
Malignant	1237 (801)	241 (267)	41 (84.1)	2.5 (0.9)
Benign	57 (15)	47 (24)	8.6 (7.1)	0.7 (0.4)
Healthy women	11 (6)	35 (14)	4.7 (4.9)	0.7 (0.3)
Comparisons	p values*			
Malignant vs. benign	<0.01	< 0.01	<0.01	<0.01
Malignant vs. healthy women	<0.01	<0.01	<0.01	<0.01
Benign vs. healthy women	0.95	0.84	0.26	0.99
Epithelial tumors (87 cases) and 150 healthy women)				
Carcinoma	1893 (1332)	373 (384)	60.4 (81.1)	4.0 (4.9)
Borderlines	28 (30)	47 (23)	9.3 (9.6)	0.4 (0.6)
Benign	20 (14)	61 (25)	10.7 (8.4)	0.7 (0.4)
Healthy women	11 (6)	35 (14)	4.7 (4.9)	0.7 (0.3)
Comparisons	p values*			
Carcinoma vs. borderline	<0.01	0.01	0.01	0.001
Carcinoma vs. benign	<0.01	0.01	0.01	0.01
Carcinoma vs. healthy women	<0.01	0.01	0.01	0.01
Borderline vs. benign	0.99	0.99	0.99	0.98
Borderline vs. healthy women	0.99	0.99	0.85	0.99
Malignant ovarian tumor (51 cases) and 150 healthy women				
Epithelial	1893 (1332)	373 (384)	60.4 (81.1)	4.0 (4.9)
Stromal	148 (99)	32 (8)	10 (8.4)	0.4 (0.4)
Germinative	176 (192)	53 (52)	13.7 (20.6)	0.4 (0.2)
Healthy women	11 (6)	35 (14)	4.7 (4.9)	0.7 (0.3)
Comparisons	p values*			
Epithelial vs. stromal	0.03	0.0003	<0.01	0.009
Epithelial vs.germinative	0.07	0.004	<0.01	0.02
Epithelial vs. healthy women	<0.01	<0.01	<0.01	<0.01
Stromal vs.germinative	0.99	0.99	0.98	0.99
Stromal vs. healthy women	0.99	0.99	0.85	0.99
Germinative vs. healthy women	0.99	0.99	0.64	0.99

IQR= interquartile range

* p values calculated with the Tukey's HSD (Honestly Significant Difference) test

Table 4: Mean serum marker concentrations in women with carcinomas as related to disease stage

Group	Mean serum concentrations (interquartile range)							
	CA125 (IQR) Unit= U/mL		HE4 (IQR) Unit= pmol/L		ROMA (IQR) Unit= probability		Mesothelin (IQR) Unit= nmol/L	
Carcinoma (37 cases) and 150 healthy women								
Stage I/II	341	(160)	214	(117)	32.4	(45.8)	1.0	(0.8)
Stage III/IV	3076	(2280)	495	(412)	81.8	(14.6)	6.2	(7.2)
Healthy women	11	(6)	35	(14)	4.7	(4.9)	0.7	(0.3)
Comparisons	p values*							
Stage I/II vs. stage III/IV	<0.01		0.0002		<0.01		<0.01	
Stage I/II vs. healthy women	<0.01		<0.01		<0.01		0.08	
Stage III/IV vs. healthy women	<0.01		<0.01		<0.01		<0.01	
Stage I/II Carcinomas and benign ovarian tumor (133 cases) and 150 healthy women								
Stage I/II carcinoma	341	(160)	214	(117)	32.4	(45.8)	1.0	(0.8)
Benign ovarian tumor	57	(15)	47	(24)	8.6	(7.1)	0.7	(0.55)
Healthy women	11	(6)	35	(14)	4.7	(4.9)	0.7	(0.3)
Comparisons	p values*							
Stage I/II carcinoma vs. benign ovarian tumor	<0.01		<0.01		<0.01		0.06	
Stage I/II carcinoma vs. healthy women	<0.01		<0.01		<0.01		0.08	
Benign ovarian tumor vs. healthy women	0.24		0.61		0.04		0.96	

IQR= interquartile range

* p values calculated with the Tukey's HSD (Honestly Significant Difference) test

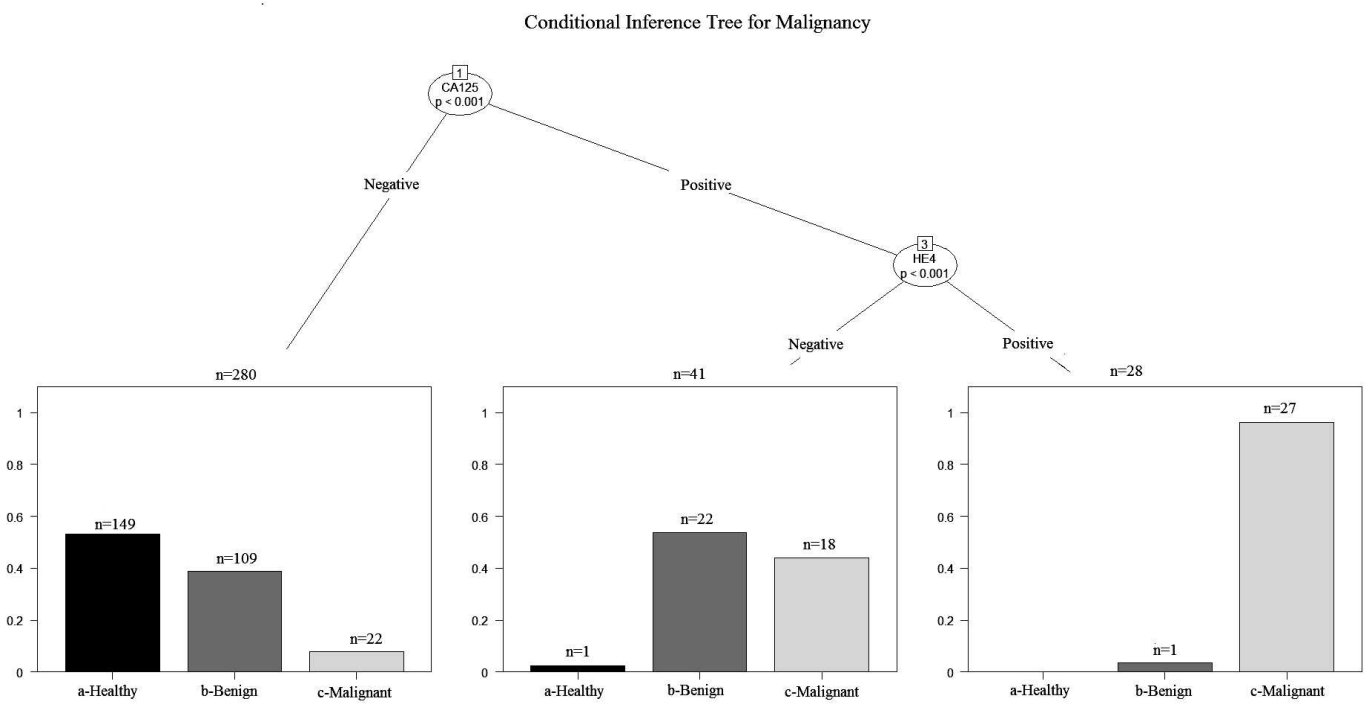

Figure 1: Conditional inference tree for the diagnoses (malignant tumors, benign tumors and healthy women) based on a recursive partitioning regression model. Only significant associations are displayed in the tree, at branch bifurcations. Variables included in the model were: age, CA125, HE4 and mesothelin levels. Note that, although included in the model, age and mesothelin do not appear in the tree because no significant association between age and mesothelin with the diagnoses was found.

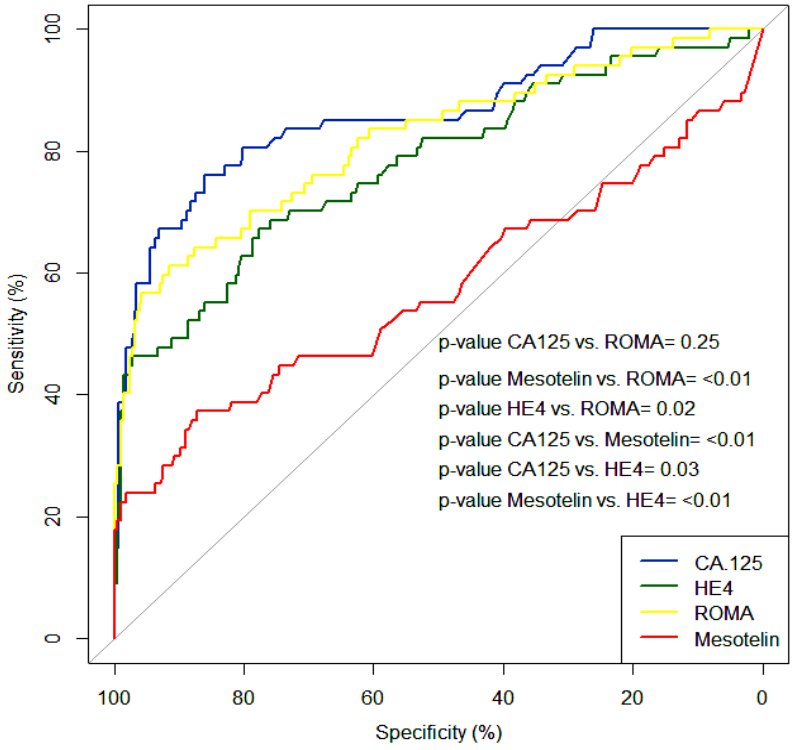

Figure 2: Receiver–operating characteristics curve analysis of CA125, HE4, mesothelin and ROMA index performance in discerning women with malignant tumors from those with benign tumors or healthy women. The areas under the curves are: CA125 = 0.86; HE4= 0.78; mesothelin= 0.57; ROMA Index= 0.82. The pairwise comparisons of the AUC for the serum markes were performed using U-Statistics theory and asymptotic normality comparisons as proposed by DeLong et al. (1988).

3.2. Artigo 2

Article title: Symptoms, CA125 and HE4 for the preoperative prediction of ovarian malignancy in Brazilian women with ovarian masses
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Symptoms, CA125 and HE4 for the preoperative prediction of ovarian malignancy in Brazilian women with ovarian masses.

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Abstract

Objective: To evaluate whether the presence of specific self-reported symptoms can be used in association to the expression of CA125, HE4 and ROMA index for the preoperative prediction of ovarian malignancy in Brazilian women with adnexal masses. **Subjects and methods:** For this cross sectional study, 178 women with ovarian tumors (61 with malignant tumors and 117 with benign tumors) and 150 healthy women were included. All women filled a self-reported symptom questionnaire. Blood samples were obtained for serum quantification of CA125 and HE4. Clusters of symptoms and isolated symptoms were identified: abdomen (abdominal bloating and/or increased abdominal size*)*; pain *(*pelvic, back and/or abdominal pain); leg swelling; digestion (indigestion and/or nauseas/vomiting); eating (unable to eat normally and/or feeling full quickly); able to feel abdominal mass; miscellaneous (fatigue and/or difficulty breathing); and bladder (urinary urgency and/or frequent urination). **Results:** The proportion of women with each of the clusters of symptoms and isolated symptoms decreased significantly from the group of women with malignant tumors to that with benign tumors and from this group to the healthy women group (p for trends in all comparisons <0.01). After a multivariate analysis the association that contributed the most to the detection of malignant ovarian tumors was that of the abdomen cluster, CA125 expression, pain cluster and weight loss. **Conclusion:** Specific symptoms were significantly higher in women with malignant ovarian tumors and may be used along with CA125 to select women with ovarian malignancy among those with adnexal masses.

Keywords: specific symptoms, ovarian tumors, CA125, HE4, ROMA, prediction of malignancy.

Introduction

Each year, close to 255.000 new cases of ovarian cancer are diagnosed around the world, it is the 7th most common type of cancer diagnosed in women. It has, however, the highest mortality rate among gynecological cancers leading to around 140.000 deaths per year [1]. The incidence of ovarian cancer is higher in industrialized countries, although the largest number of cases is concentrated in developing countries (96.700 vs 107.500). In Latin America, the 8/100.000 incidence is close to that of developed countries, which is of 10/100.000 women. It is expected that 6.190 ovarian cancer cases will be diagnosed in Brazil in 2012, with an estimated risk of 6:100.000. Not considering non-melanoma skin cancer, ovarian cancer is the seventh most frequent cancer in Brazilian women [2].

The preoperative diagnosis of malignancy is hard to be performed and one in every 10 women will be operated because of an adnexal tumor in her lifetime; among these women approximately 17% will be diagnosed with a malignant ovarian tumor [3]. Serum analysis of tumor markers and ultrasound were the traditional approach to women with adnexal masses and CA125 measurement is an important component in the workup of a woman with an adnexal mass [4, 5]. Recently, HE4 (human epididymis protein 4) has been proven to be more sensitive for the detection of ovarian cancer compared to CA125 alone [6]. Moore *et al*. [7] developed a Risk of Ovarian Malignancy Algorithm (ROMA) index to classify patients with a pelvic mass into high-risk or low-risk groups for ovarian carcinoma, which combines serum levels of CA125 and HE4 along with menopausal status in a logistic regression model. ROMA index has performed better than other Risk of Malignancy Indexes (RMI) [4]. Nowadays, pelvic ultrasound and serum quantification of CA125 and HE4 are the indicators that have best sensitivity and specificity in the preoperative diagnosis of possibly malignant tumors [8, 9, 10].

Recently, symptoms that could help in the selection of women with higher risk of malignancy are the subject of many studies. Due to the anatomical position of the ovary deep in the abdominal cavity it was supposed that in early stages, ovarian cancer would not cause symptoms. However, this "silent killer" concept has been challenged and it seems it should be discarded [11]. Studies have shown that women with ovarian cancer in any stage do have symptoms, up to 36 months before diagnosis [12,13]. These symptoms are usually underestimated by women and their consultants. However, when a women over 50 years of age is examined, if she repeatedly complains of abdominal distension, gastrointestinal or urinary habit change, abdominal or pelvic pain, or increase of the abdominal circumference - to which an acute condition is excluded such as gastroenteritis, etc - ovarian cancer should be considered among the differential diagnosis. It is important to understand that these symptoms are vague and still not fully understood.

Recently, some groups of researchers are conducting trials that aim at establishing which would be the specific symptoms of ovarian cancer and what would be the best strategy does diminish delays in diagnosis. Selection of symptomatic patients seems to be the most promising way of detecting ovarian cancer while the disease has a smaller volume which is of easier therapeutic control [14, 15, 11, 16]. Therefore, the objective of this study was to evaluate whether the presence of specific self-reported symptoms can be used in association to the expression of CA125, HE4 and ROMA index for the preoperative prediction of ovarian malignancy in Brazilian women with adnexal masses.

Subjects and methods

This was a cross-sectional study with prospective data collection. This study was approved by the international review board of the Unicamp School of Medicine under

number 1092/2009 and an informed consent was obtained from all participants. All women with adnexal masses, attending the hospital of the Department of Obstetrics and Gynecology of the Unicamp School of Medicine were invited to participate in the study. A control group of healthy women attending menopause and family planning clinics at the same hospital were selected. After an explanation about the study research methods and purpose all women answered a survey regarding specific symptoms according to [17]. There were also collected data on age and body mass index. Peripheral blood was collected for serum measurements of CA125 and HE4. We included in this study 199 women with adnexal masses operated between January 2010 and January 2012, and 150 healthy women who had consultations within the same time period.

Symptoms

The women enrolled completed an identical survey asking about the occurrence of 22 symptoms that have been reported to be related to ovarian cancer by Goff et al. [17]. The survey evaluated the presence, frequency and duration of pelvic pain, abdominal pain, back pain, indigestion, unable to eat normally, feeling full quickly, nausea or vomiting, weight loss, abdominal bloating, increased abdomen size, able to feel abdominal mass, urinary urgency, frequent urination, constipation, diarrhea, menstrual irregularity, bleeding after menopause, pain during intercourse, bleeding with intercourse, fatigue, leg swelling, difficulty breathing and others. The survey was originally designed in English and was submitted to a Portuguese translation, which included two forward translations, one reconciled version and a back translation of the reconciled version. Initially, the patient was questioned about the presence or absence of a symptom. If present, the severity of each symptom along with its frequency and duration was evaluated. The frequency was

reported with respect to the number of days per month, classified as: <1, 1-2, 3-6, 7-12, 13-19 or >20 days/month. The duration was reported with respect to how long did the symptom persist, then it was categorized in how many of the previous twelve months, < 1, 1-2, 3-4, 5-6, 7-9, 10-12, >12. We considered a symptom positive if it occurred more than 12 times per month and up to one year, regardless of this severity [17, 18]. In the surgical population all women were surveyed prior to surgery, before they knew their histological diagnosis.

Serum samples and marker assays

Blood samples were collected from all patients and stored in Serum Separator Tubes. They were allowed to clot for at least 30 minutes before centrifugation. The blood samples were centrifuged 1300g for 10 min, and serum was aliquoted and stored at -80°C until analysis. Automated analysis of CA125 was performed by solid phase chemiluminescence using the OM-MA test (Siemens Medical Solutions Diagnostics, Tarrytown, USA) according to the manufacturer's instructions and using their reagents and equipment. Values were expressed in units per milliliter (U/mL). The level of serum HE4 was determined using the HE4 enzyme immunometric assay Kits (EIA) (Fujirebio Diagnostics, Göteborg, Sweden) based on the direct sandwich technique, solid-phase immunoassay according to the manufacturer's instructions and using their reagents and equipment. Values were expressed in picomoles per liter (pmol/L). The Risk of Ovarian Malignancy Algorithm (ROMA) uses the results for HE4 and CA125 to generate a predictive index (PI) for EOC, calculated by the formulas proposed by Moore at al. [19] for pre menopausal and post menopausal women. ROMA index was used to stratify women into high-risk or low-risk groups for having a pelvic mass that is malignant or benign respectively.

Surgery and pathological assessment

Surgery for diagnosis and/or treatment was performed at the hospital of the Department of Obstetrics and Gynecology of Faculty of Medical Sciences (FCM/Unicamp) and the techniques and surgical procedures were chosen and performed according to medical indication. The mean time elapsed between blood collection and surgery ranging from 24 h or less for emergency procedures to a maximum of 120 days. The gold standard was the histopathologic diagnosis of surgical specimens, all performed in the Department of Pathologic Anatomy of the FCM/Unicamp following the guidelines of the World Health Organization International Classification of Ovarian Tumors [20]. For statistical purposes, borderline tumors were classified as malignant.

Statistical analysis

Data were entered into a Microsoft Excel (Microsoft Corp., Redmond, WA, USA) spreadsheet and analyzed with the R Environment for Statistical Computing Software® [21]. All statistical calculations were performed using 95% confidence interval (CIs), considering $P < 0.05$ as significant. Women were classified into benign and malignant groups according to tumor histologic diagnosis. The sample size was calculated on the basis of the difference in symptom prevalence derived from previous studies, with 5% significance levels, 80% statistical power and 12% error limits for the sensitivity: the minimal number of women with malignant tumors would be 54, and based on the prevalence of malignancy, 112 women with benign tumors would be needed for discrimination. The number of controls (healthy women) was estimated at 122, totaling 276 women.

Firstly, we compared the key clinical features of women with ovarian malignant tumors, benign tumors and healthy women. We also compared the mean and standard

deviation of age, body mass index, serum CA125 and HE4 levels and ROMA index. Chi-square test for categorical variables and Kruskal-Wallis test for continuous variables were used for statistical analysis. We next calculated the proportion of women with each of the 22 specific symptoms in the groups of women with ovarian malignant tumors, benign tumors and healthy women. We considered a symptom positive if it occurred more than 12 times per month and up to one year, regardless of this severity. The proportions were pairwise compared using chi-square or the Fisher exact test where appropriate.

The specific symptoms which applied to the entire cohort and for which the frequency had been ascertained were further subjected to Ward's Hierarchical Clustering Method. The following specific symptoms were thus not included in Ward's model: menstrual irregularity, bleeding after menopause, pain during intercourse, bleeding with intercourse (because they depend on menopausal status and sexual activity); constipation and diarrhea (which appeared very rarely) and weight loss because frequency is not applicable to that symptom. The 16 remaining symptoms were included in the Ward method that allows the formation of statistically significant agglomerates of symptoms, which were depicted into a Euclidian plane: related symptoms appear close to each other in Figure 1, the closer they are, the more related to each other. The Ward agglomerative method was able to define 6 different clusters of symptoms as following: abdomen (abdominal bloating and/or increased abdominal size*)*; pain *(*pelvic, back and/or abdominal pain); digestion (indigestion and/or nauseas /vomiting); eating (unable to eat normally and/or feeling full quickly); miscellaneous (fatigue and/or difficulty breathing) and bladder (urinary urgency and/or frequent urination). The two following symptoms, leg swelling and able to feel abdominal mass, remained as isolated symptoms. We evaluated the trend in proportion of women with each cluster of symptoms or isolated symptoms in the groups of women with malignant tumors, benign

tumors and healthy women using the chi-squared test for trend in proportions. Next, we calculated the Odds Ratios (OR) with 95% Confidence Intervals (CI95%) and pairwise multivariate comparisons of the proportions of women with each cluster of symptoms or isolated symptoms using the likelihood-ratio test (LR-Test). We used a recursive partitioning algorithm, based on a linear regression model as described by Hothorn *et al*.[22] to confirm the contribution of patient age, each cluster of symptoms or isolated symptoms, menopausal status, weight loss, CA125 and HE4 level to differentiating women with malignant tumors, benign tumors and healthy women. The aforementioned variables were included in the recursive partitioning regression model, and a conditional inference tree was generated. Conditional inference tree estimates a regression relationship by binary recursive partitioning in a conditional inference framework. Branches of the generated inference tree bifurcate when a statistically significant association is detected ($P<0.05$).

Results

Among the 199 women with adnexal tumors, 132 had benign tumors, 117 of ovarian origin and 67 malignant, 61 of ovarian origin. Among the 61 ovarian cancer women, 33 were in stage I, 5 in stage II, 23 in stages III / IV (date not shown). Both in malignant and benign ovarian tumors, the epithelial type were predominant. Among the benign ovarian tumors, there was also a high prevalence of mature teratomas and fibromas (Table 1). The mean age was significantly higher in women with malignant tumors (50.8 $\pm$ 20.5 years) compared with the control group (44.4 $\pm$ 12.6 years) ($p < 0.02$). There were a higher proportion of postmenopausal women in the malignant tumors group when compared with the benign tumors group. There were no differences in BMI between the groups of women studied. Expression of CA125, HE4, and the values of ROMA were significantly

higher among women with malignant ovarian tumors compared with benign tumors and controls ($p < 0.01$). Among women with benign ovarian tumors and controls the expression of the markers was similar.

In univariate analysis, when symptoms were evaluated, we found that women with malignant tumors showed a high frequency of pelvic pain, abdominal pain, back pain, unable to eat normally, feeling full quickly, indigestion, weight loss, abdominal bloating, increased abdominal size, able to feel abdominal mass, urinary urgency, frequent urination and fatigue. Univariate analysis found that the majority these symptoms were significantly more frequent in women with malignant tumors compared with those with benign tumors and the control group and when comparing women with benign tumors and those in the control group (see individual significance of each symptom in Table 3).

In Figure 1, which shows the cluster dendogram based on the Ward agglomerative method, clusters of symptoms were identified when symptoms agglomerated at the lowest level of the cluster dendogram. Symptoms that did not agglomerate at the lowest level of the cluster dendogram were considered as isolated symptoms. The following clusters of symptoms and isolated symptoms were thereby identified: abdomen (abdominal bloating and/or increased abdominal size); pain (pelvic, back and/or abdominal pain); leg swelling; digestion (indigestion and/or nauseas/vomiting); eating (unable to eat normally and/or feeling full quickly); able to feel abdominal mass; miscellaneous (fatigue and/or difficulty breathing); bladder (urinary urgency and/or frequent urination).

Table 4 shows the proportion of women with each of the clusters of symptoms/isolated symptoms in the groups of women with malignant tumors, benign tumors and healthy women. The proportion of women with each of the clusters of symptoms and isolated symptoms decreased significantly from the group of women with malignant

tumors to that with benign tumors and from this group to the healthy women (p for trends in all comparisons= <0.01).

Table 5 shows the multivariate pairwise comparisons of the proportions of women with each of the clusters of symptoms or isolated symptoms in the group of women with malignant tumors, benign tumors and healthy women. The proportion of women with the abdomen cluster of symptoms was higher in women with malignant tumors compared to those with benign tumors (p=0.03) and healthy women (p<0.01), and in those with benign tumors compared to healthy women (P<0.01). The proportion of women with the pain cluster of symptoms was significantly higher in women with malignant tumors compared to healthy women (p<0.01) and in women with benign tumors compared to healthy women (p=0.02). The proportion of women with the eating cluster of symptoms was significantly higher in women with malignant tumors compared to women with benign tumors (p=0.01) and healthy controls (p<0.01). The bladder cluster of symptoms was significantly more prevalent in women with benign tumors compared with healthy women (p=0.03).

Figure 2 shows the results of the conditional inference tree in which patient age, clusters of symptoms and isolated symptoms, menopausal status, weight loss, CA125 and HE4 levels were included. The abdomen cluster of symptoms had the greatest capacity to discriminate between women with malignant tumors, benign tumors and healthy women, as shown by the first branch bifurcation of the tree. In women without the abdomen cluster of symptoms, CA125 levels contributed further (p<0.01) to the differentiation among malignant tumors, benign tumors and the control group. In women without the abdomen cluster of symptoms and with normal CA125 levels, the pain cluster of symptoms was of statistical significance in this differentiation (p=0.012), and for those without those symptoms, weight loss also had a significant (p=0.039) differentiation contribution.

Discussion

In this sample of Brazilian women, we found that among those with ovarian malignancy most patients reported at least one symptom, more than 12 times a month, for up than a year before consultation. Although these symptoms were also reported in women with benign tumors and in the control group, the presence of pelvic pain, abdominal pain, back pain, unable to eat normally, filling full quickly, indigestion, nausea or vomiting, abdominal bloating, increased abdomen size, able to feel abdominal mass, bleeding after menopause, fatigue and difficult breathing was significantly higher among women with ovarian cancer compared with those with benign tumor. Being several correlated symptoms, we evaluated the possible groupings and identified six clusters and two isolated symptom by the technique of Ward: abdomen (abdominal bloating and/or increased abdominal size); pain (pelvic, back and/or abdominal pain); digestion (indigestion and/or nauseas/vomiting); eating (unable to eat normally and/or feeling full quickly); miscellaneous (fatigue and/or difficulty breathing); bladder (urinary urgency and/or frequent urination). The two symptoms that remain isolated were leg swelling and able to feel abdominal mass. The proportion of women with the abdomen, pain and eating clusters of symptoms was higher in women with ovarian malignant tumors. The bladder cluster of symptoms was significantly more prevalent in women with benign tumors compared with healthy women. Overall, the abdomen cluster of symptoms had the greatest capacity to discriminate between women with malignant tumors, benign tumors and healthy women. CA125 levels, pain cluster and weight loss contributed further to the differentiation among malignant tumors, benign tumors and the control group.

Many studies regarding the symptoms associated with ovarian cancer have been reported in the last decade, and now one cannot consider ovarian cancer as an asymptomatic

disease [23, 17, 18, 15]. Women with ovarian cancer have multiple symptoms; however, usually these are not gynecological. Being essentially general symptoms, both women and physicians tend to underestimate these symptoms. Doctors often treat women as irritable bowel syndrome, stress, depression or gastritis, months before they detect an ovarian cancer [13]. The underestimation of symptoms by women and doctors may contribute to ovarian cancer being diagnosed in advanced stages. In our sample, all women with malignancies reported some kind of symptom, being characterized as relevant those that appeared more than 12 times a month in the last year before consultation [17]. This sample of Brazilian women presented thus data consistent with studies in other populations: after interviewing 1725 women with ovarian cancer in the United States and Canada, Goff et al.[23] found out that 95% of them reported having had symptoms 3 to 6 months before looking for a doctor, regardless of the disease stage - in this study, the most common symptoms were increased abdominal volume (77%), gastrointestinal (70%) , pain (58%), constitutional (50%), bladder (34%) and pelvic (26%).

In our study, we found significant differences between the mean values of CA125, HE4 and ROMA among women with malignant tumors, benign tumors and controls. These markers undoubtedly play an important role in the differentiation of adnexal masses, although its significance regarding changes in medical practice is still subject of several studies[8]. As a screening method, no marker is recommended in asymptomatic women without a family history of breast or ovarian cancer or BRCA1 and BRCA2 mutation carriers of. Recently, however, the American College of Obstetrics and Gynecology issued recommendation to offer pelvic examination, serum CA125 and transvaginal ultrasound for women with symptoms of ovarian cancer [24]. In our study, after multivariate analysis, CA125 levels contributed further to the differentiation among malignant tumors, benign

tumors and the control group. Thus there is a great expectation to evaluate the prevalence of malignant tumors in women who have symptoms related to ovarian cancer according to levels of CA125 and other markers. Andersen *et al.* [18] in a prospective study comparing 74 women with ovarian cancer and 137 healthy women found out that either CA125 or HE4, when combined with the symptom index, detected 91.9% of the cases of malignancy. However, based on our results, there is not yet enough evidence to recommend the use of HE4 to discriminate women with adnexal masses.

Our data demonstrated that symptoms may be used even to detect early stage ovarian disease. In the present study, 62% of the patients had stage I/II disease, regardless of the histological type of the tumor. Rossing *et al*. [25] demonstrated that the symptom index was positive in 62.3% of women with early stage disease. However, in their study, women were surveyed on average 9 months after diagnosis, whereas in our study we surveyed the women before surgery. One limitation of our study is that we have not analyzed pre and postmenopausal women separately. Of course, in our analyses, symptoms which applied only to pre- or postmenopausal women and those applicable only to sexually active women were not included in the multivariate models. Unfortunately, this approach is not sufficient to rule out the selection bias since, for example, pelvic pain, which was significantly associated with malignancy, is frequently reported by young women with endometrioma [26].

In this sample of Brazilian women, we found that among those with ovarian malignancy most patients reported at least one symptom more than 12 times a month, for up to a year before consultation. Although these symptoms were also reported in women with benign tumors and in the control group, the proportions of women with the abdomen, pain and eating clusters of symptoms as well as weight loss were higher in

women with ovarian malignant tumors. Overall, the abdomen cluster of symptoms had the greatest capacity to discriminate between women with malignant tumors, benign tumors and healthy women while CA125 levels, pain cluster and weight loss contributed further to the differentiation among malignant tumors, benign tumors and the control group. It is quite clear that these symptoms are not unique of ovarian cancer and are frequently related to other diseases. Due to the rarity of ovarian cancer and the high prevalence of these symptoms in the general population, the predictive value of these symptoms is reduced as a screening method. However, based on our results, it is clear that the evaluation of specific symptoms should be recommended in the clinical setting for pre operative discrimination of Brazilian women with adnexal masses.

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Table 1: Distribution of 199 women according to histological type

BENIGN TUMORS	n	(%)	MALIGNANT TUMORS	n	(%)
OVARY			**OVARY**		
			EPITHELIAL		
EPITHELIAL			**A)MALIGNANT**		
Serous cystadenoma and/or Serous cystadenofibroma	22	(17)	Serous adenocarcinoma	18	(27)
Mucinous cystadenoma	10	(8)	Endometrioid adenocarcinoma *	9	(13)
Brenner tumor	4	(3)	Mucinous adenocarcinoma *	4	(6)
			Clear cell adenocarcinoma	3	(4.5)
			Mixed adenocarcinoma	2	(3)
			Carcinosarcoma	1	(1.5)
EPITHELIAL+SEX CORD STROMAL			**B)BORDERLINE**		
Brenner tumor + fibroma	2	(1.5)	Serous	3	(4.5)
Serous cystadenoma + fibroma	1	(0.8)	Mucinous intestinal pattern	4	(6)
Serous cystadenofibroma + fibroma	1	(0.8)	Seromucinous	3	(4.5)
SEX CORD AND STROMAL			**SEX CORD AND STROMAL**		
Fibroma	14	(11)	Granulosa cell tumor	6	(9)
Fibrothecoma	3	(2)	Sertoli-Leydig	1	(1.5)
Sclerosing stromal tumor	1	(0.8)	Ginandroblastoma	1	(1.5)
Leiomyoma	2	(1.5)			
GERM CELL TUMOR			**GERM CELL TUMOR**		
Mature teratoma	29	(22)	Immature teratoma	3	(4.5)
			Mature teratoma with carcinomatous transformation	1	(1.5)
			Dysgerminoma	2	(3)
NON- NEOPLASTIC OVARIAN			**OVARIAN METASTASIS**		
Endometrioma	12	(9)	From endometrial cancer	2	(3)
Ovarian edema	1	(0.8)	From intestinal cancer	1	(1.5)
Functional cysts	11	(8)	From unknown primary site	1	(1.5)
Hemorragic cysts	2	(1.5)			
Ovarian abscess	2	(1.5)			
TOTAL OVARY	**117**		**TOTAL OVARY**	**65**	
EXTRA-OVARIAN			**EXTRA-OVARIAN**		
Hydrosalpinx	3	(2)	Extra ovarian serous adenocarcinoma	1	(1.5)
Adenomyoma or para-uterine leiomyoma	4	(3)	Uterine leiomyosarcoma	1	(1.5)
Uterine leiomyoma	3	(2)			
Morgagni hydatides	2	(1.5)			
Tubarian cystadenofibroma	1	(0.8)			
Tubarian vascular congestion	1	(0.8)			
Tubarian endometrioma	1	(0.8)			
TOTAL BENIGN	**132**		**TOTAL MALIGNANT**	**67**	

*2 ovarian carcinoma had endometrial carcinoma associated, in these cases considered ovarian carcinoma as the primary tumor

Table 2. Key clinical features and serum marker levels of women with ovarian malignant tumors, benign tumors and healthy women

Characteristics	Malignant	Benign	Healthy women	p-value (malignant vs. benign)	p-value (malignant vs. healthy women)	p-value (benign vs. healthy women)
Age						
Years, mean (SD)*	50.8 (20.5)	47.86 (16.6)	44.4 (12.6)	0.45	0.02	0.18
Menopausal status[T]						
Premenopausal	23	68	79			
Postmenopausal	38	49	71	<0.01	0.05	0.37
Body mass index (BMI) – kg/m²						
Mean (SD)*	28.3 (6.3)	28.0 (5.5)	27.2 (5.0)	0.42	0.37	0.40
CA125 (U/ml)						
Mean (SD)*	1190 (3051)	57 (252)	11 (5)	< 0.01	<0.01	0.95
HE4 pmol/L						
Mean (SD)*	244 (414)	48 (78)	35 (32)	< 0.01	<0.01	0.84
ROMA index						
Mean (SD)*	40.9 (39.6)	8.6 (14.5)	4.8 (7.8)	< 0.01	<0.01	0.24
Total	**61**	**117**	**150**			

[T] p values calculated with either chi-squares[T] or the Kruskal-Wallis test*.

Table 3. Specific symptoms in women with ovarian malignant tumors, benign tumors and healthy women

	Malignant tumors (n=61)		Benign tumors (n=117)		Healthy women (150)		p-value (Malignant vs benign)	p-value (Malignant vs healthy women)	p-value (Benign vs healthy women)
Symptom	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**			
Pelvic pain	30	(50)	23	(19.7)	2	(1.3)	<0.01	<0.01	<0.01
Abdominal pain	23	(38.3)	10	(8.5)	0	(0)	<0.01	<0.01	<0.01
Back pain	17	(28.3)	10	(8.6)	0	(0)	<0.01	<0.01	<0.01
Unable to eat normally	22	(36.7)	8	(6.8)	1	(0.7)	<0.01	<0.01	0.01
Feeling full quickly	22	(36.1)	11	(9.4)	1	(0.7)	<0.01	<0.01	<0.01
Indigestion	15	(24.6)	7	(6.0)	1	(0.7)	<0.01	<0.01	0.02
Nausea or vomiting	11	(18.0)	4	(3.4)	1	(0.7)	<0.01	<0.01	0.17
Weight loss	19	(31.7)	25	(21.6)	11	(7.3)	0.19	<0.01	<0.01
Abdominal bloating	36	(60.0)	32	(27.4)	0	(0)	<0.01	<0.01	<0.01
Increased abdomen size	38	(63.3)	31	(26.7)	1	(0.7)	<0.01	<0.01	<0.01
Able to feel abdominal mass	14	(23.3)	11	(9.4)	0	(0)	0.02	<0.01	<0.01
Urinary urgency	8	(13.3)	8	(6.8)	2	(1.3)	0.25	<0.01	0.02
Frequent urination	12	(20.0)	16	(13.7)	2	(1.3)	0.38	<0.01	<0.01
Constipation	2	(3.3)	2	(1.7)	0	0	0.60	0.08	0.18
Diarrhea	3	(5.1)	1	(0.9)	0	0	0.11	0.02	0.43
*Menstrual irregularities**	1	(1.6)	1	(0.9)	1	(0.7)	1.0	0.49	1.0
*Bleeding after menopause***	6	(10.0)	1	(0.9)	0	0	<0.01	<0.01	0.43
*Pain during intercourse****	0	0	0	0	1	(0.7)	NC	1.0	1.0
Bleeding with intercourse	0	0	0	0	0	0	NC	NC	NC
Fatigue	20	(33.3)	16	(13.7)	3	(2.0)	<0.01	<0.01	<0.01
Leg swelling	6	(10.0)	11	(9.4)	1	(0.7)	1.0	<0.01	<0.01
Difficulty breathing	9	(15.0)	5	(4.3)	2	(1.3)	0.02	<0.01	0.24

* only for premenopausal women; ** only for postmenopausal women; ***only for sexually active women; NC= non-computable.

P-value: bivariate pairwise comparisons using chi-squares or the Fisher's exact test where appropriate.

Table 4. Proportion of women with each of the clusters of symptoms/isolated symptoms in the groups of women with malignant tumors, benign tumors and healthy women

Symptoms	Malignant positive/total(%)		Benign positive/total(%)		Healthy women positive/total(%)		p trend
Cluster abdomen	41/60	(68.3)	36/116	(31)	1/150	(0.7)	<0.01
Cluster pain	34/60	(56.7)	26/116	(22.4)	2/150	(1.3)	<0.01
Leg swelling	6/60	(10)	11/117	(9.4)	11/150	(0.7)	<0.01
Cluster digestion	18/61	(29.5)	10/117	(8.5)	2/150	(1.3)	<0.01
Cluster eating	27/60	(45)	14/117	(12)	2/150	(1.3)	<0.01
Able to feel abdominal mass	14/60	(23.3)	11/117	(9.4)	0/150		<0.01
Cluster miscellaneous	21/39	(35)	18/117	(15.4)	5/150	(3.3)	<0.01
Cluster bladder	14/60	(23.3)	20/117	(17.1)	3/150	(2)	<0.01

Clusters of symptoms and isolated symptoms were defined by the Ward agglomerative method: abdomen (abdominal bloating and/or increased abdominal size*)*; pain *(*pelvic, back and/or abdominal pain); leg swelling; digestion (indigestion and/or nauseas /vomiting); eating (unable to eat normally and/or feeling full quickly); able to feel abdominal mass; miscellaneous (fatigue and/or difficulty breathing); bladder (urinary urgency and/or frequent urination). P trend: calculated by chi-squared test for trend in proportions.

Table 5: pairwise comparisons of the proportions of women with each of the clusters of symptoms or isolated symptoms

Symptoms	Malignant vs Benign		Malignant vs Healthy women		Benign vs Healthy women	
	OR (IC95%)	**p (LR-Test)**	**OR (IC95%)**	**p (LR-Test)**	**OR (IC95%)**	**p (LR-Test)**
Cluster abdomen	2.6 (1.1 to 6.2)	0.03	82.7 (5.6 to 1209.3)	<0.01	23.7 (3.0 to 187.7)	<0.01
Cluster pain	2.3 (0.9 to 5.4)	0.07	14.3 (2.0 to 103.8)	<0.01	6.0 (1.1 to 32.7)	0.02
Leg swelling	0.5 (0.1 to 1.8)	0.27	4.7 (0.2 to 115.2)	0.34	5.5 (0.4 to 68.0)	0.16
Cluster digestion	0.9 (0.3 to 2.9)	0.81	0.1 (0 to 6.4)	0.29	0.75 (0.08 to 7)	0.80
Cluster eating	4.3 (1.4 to 13.3)	0.01	47.4 (2.8 to 798.7)	<0.01	2.44 (0.31 to 19.2)	0.39
Able to feel abdominal mass	2.0 (0.7 to 5.3)	0.2	NC	NC	NC	0.01
Cluster miscellaneous	1.0 (0.3 to 2.7)	0.93	0.3 (0.1 to 6.2)	0.44	1.54 (0.37 to 6.4)	0.55
Cluster bladder	0.4 (0.2 to 1.2)	0.09	1.2 (0.1 to 30.3)	0.90	4.42 (1.1 to 18.1)	0.03

NC = non-computable

Clusters of symptoms and isolated symptoms were defined by the Ward agglomerative method: abdomen (abdominal bloating and/or increased abdominal size*)*; pain *(*pelvic, back and/or abdominal pain); leg swelling; digestion (indigestion and/or nauseas /vomiting); eating (unable to eat normally and/or feeling full quickly); able to feel abdominal mass; miscellaneous (fatigue and/or difficulty breathing); bladder (urinary urgency and/or frequent urination). OR (Odds Ratio) with 95% Confidence Intervals (CI95%) and pairwise multivariate comparisons of the proportions of women with each of the clusters of symptoms using the likelihood-ratio test (LR-Test).

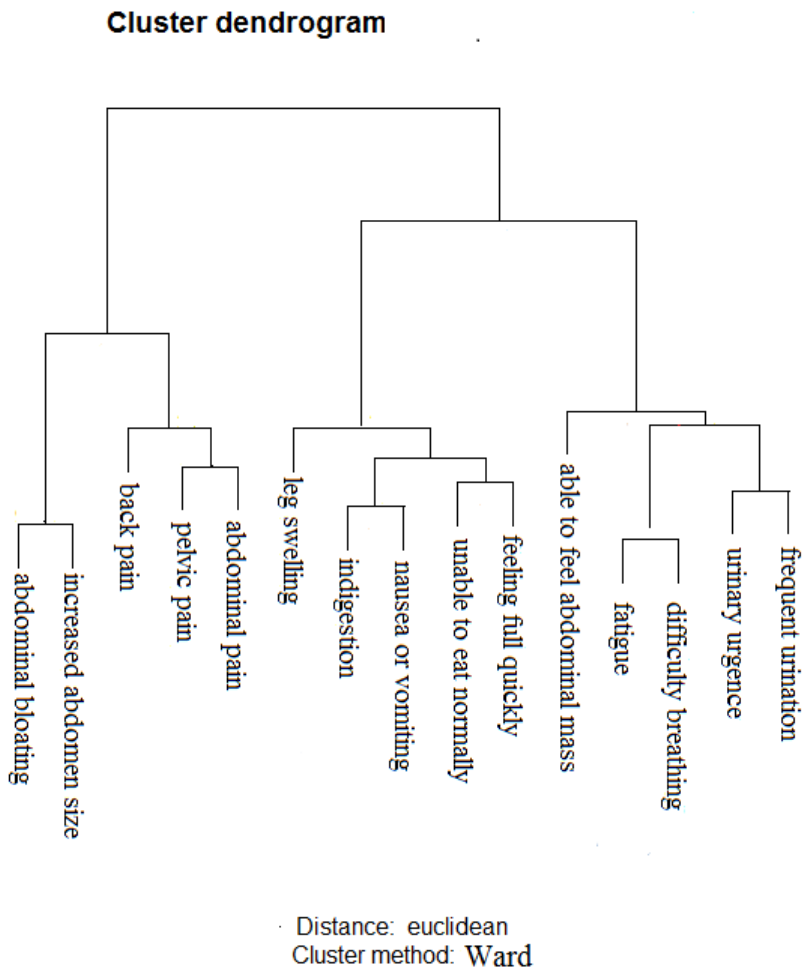

Figure 1: Ward agglomerative method for hierarchical clustering. The following Clusters of symptoms and isolated symptoms were defined by the Ward agglomerative method: abdomen (abdominal bloating and/or increased abdominal size); pain (pelvic, back and/or abdominal pain); leg swelling; digestion (indigestion and/or nauseas /vomiting); eating (unable to eat normally and/or feeling full quickly); able to feel abdominal mass; miscellaneous (fatigue and/or difficulty breathing); bladder (urinary urgency and/or frequent urination).

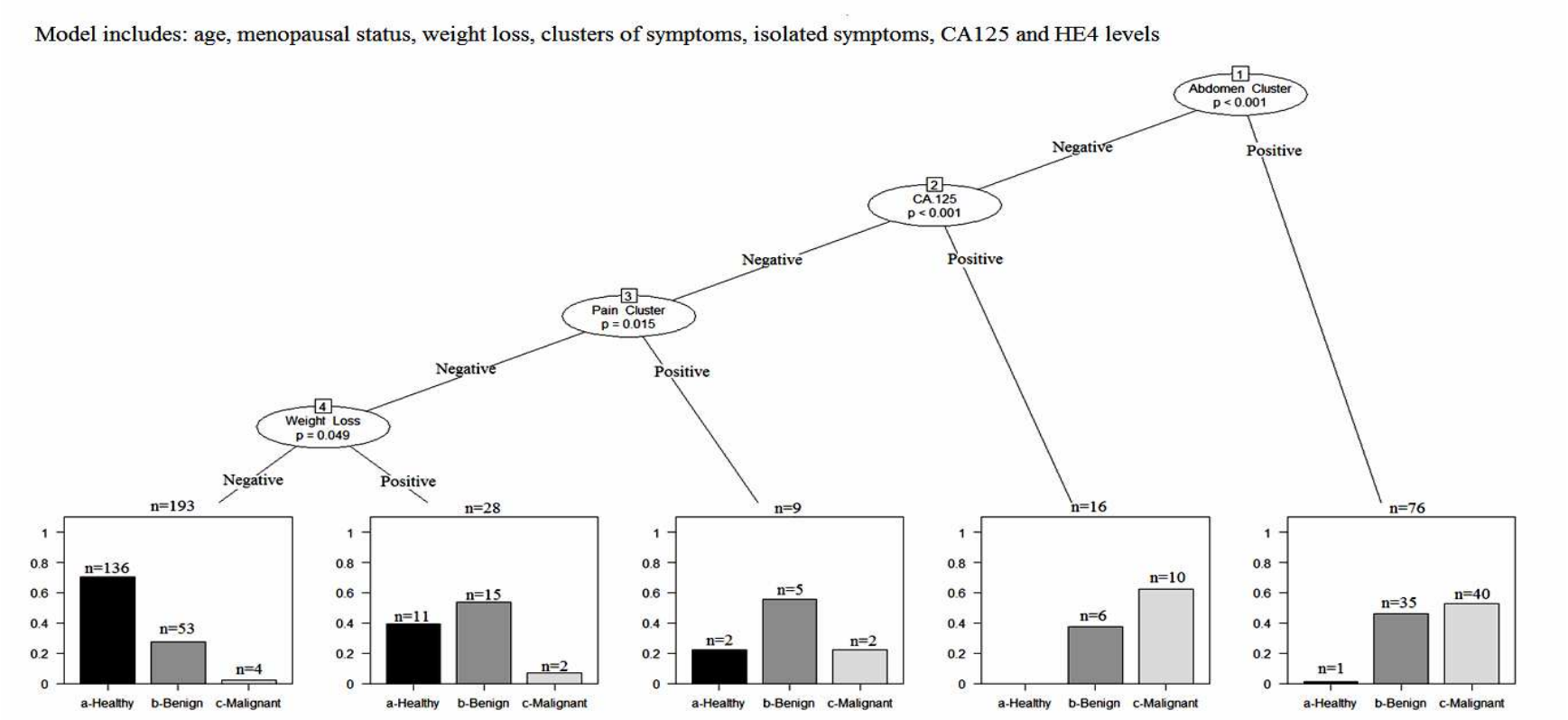

Figure 2: Conditional inference tree for the diagnoses (malignant tumors, benign tumors and healthy women) based on a recursive partitioning regression model. Only significant associations are displayed in the tree, in branch bifurcations. Variables included in the model were: patient age, clusters of symptoms and isolated symptoms [abdomen (abdominal bloating and/or increased abdominal size); pain (pelvic, back and/or abdominal pain); leg swelling; digestion (indigestion and/or nauseas/vomiting); eating (unable to eat normally and/or feeling full quickly); able to feel abdominal mass; miscellaneous (fatigue and/or difficulty breathing); bladder (urinary urgency and/or frequent urination)], menopausal status, weight loss, CA125 and HE4 levels. Note that, although all variables listed above were included in the model, only the abdomen (cluster of symptoms), CA125 level, pain (cluster of symptoms) and weight loss contributed significantly to the differentiation of women with malignant tumors, benign tumors and healthy women.

4. Discussão

A proposta deste estudo foi avaliar a expressão dos marcadores séricos e a presença de sintomas específicos em mulheres com ou sem massas anexiais. Dessa forma, no primeiro artigo, demonstrou-se que os níveis dos marcadores séricos mesotelina, CA125 e HE4 foram significativamente mais altos em mulheres com tumores malignos quando comparados aos dos tumores benignos e mulheres saudáveis. A acurácia do CA125 na detecção de tumores malignos foi maior que a do HE4 e da mesotelina. Níveis de mesotelina elevados foram observados somente em mulheres com carcinoma primários ou metastáticos. Os níveis de HE4 foram negativos em todas as mulheres com tumor maligno que apresentaram níveis de CA125 negativo. Consequentemente, o índice ROMA foi menos sensível que o CA125 como um marcador isolado.

Neste estudo, mulheres com tumores malignos da linhagem germinativa e do estroma ovariano raramente apresentaram níveis de CA125 e HE4 positivos. Estes dados estão de acordo com os de outros estudos, mostrando que a HE4 auxilia na detecção de carcinomas ovarianos (14, 43). Assim, quando consideramos

as mulheres com carcinomas, aquelas com o tipo seroso foram as que apresentaram níveis de mesotelina, CA125 e HE4 mais alto. Todavia, mulheres com carcinomas mucinosos não expressaram a mesotelina, o CA125 e a HE4, independente do estádio da doença. A expressão da mesotelina e HE4 poderia trazer benefício, pois esse subtipo histológico não expressa o CA125; porém esses marcadores também são negativos neste tipo de tumor (44, 45).

Nas mulheres com carcinomas, comparando a expressão dos marcadores entre os estádios I/II e III/IV, os níveis dos três marcadores foram significantemente mais elevados nos estádios avançados. Mulheres com carcinomas estádios I/II apresentaram níveis significantemente maiores de CA125 e HE4 (enquanto o nível de mesotelina foi normal) comparados aos das mulheres com tumores benignos e mulheres saudáveis. A expressão da HE4 seria relevante nos carcinomas em estádios iniciais, pois, nesses casos, o CA125 é expresso em metade das mulheres com tumores malignos. Entretanto, a maior parte dos estudos ainda observa que o CA125 é melhor para detectar os carcinomas nos estádios iniciais, sendo a HE4 expressa essencialmente nos carcinomas em estádios avançados (48). Avaliando especificamente a mesotelina, nos estudos de Abdel-Azeez et al. (46) e Rdzanek et al. (47), o nível de mesotelina foi significativamente maior nos estádios III/IV comparado ao dos estádios I/II.

Mulheres com tumores borderlines raramente apresentaram níveis elevados dos marcadores: apenas 3 das 10 (30%) das mulheres com tumores borderlines deste estudo expressaram o CA125, e nenhuma expressou a HE4 ou a mesotelina.

Vários estudos vêm demonstrando que a expressão do CA125, HE4 e ROMA não diferencia os tumores borderlines dos tumores benignos (41, 43, 49).

Neste estudo, em mulheres na pré-menopausa, o HE4 desempenha o importante papel de predizer quais massas anexiais são benignas. Nessas mulheres, muitas condições benignas, como os endometriomas (8 das 12), apresentaram níveis elevados do CA125, e o HE4 foi positivo em apenas 1 caso. Holcomb et al. (50), observaram que de 229 mulheres americanas com massa anexial na pré-menopausa, 85% eram benignas: entre essas, o CA 125 estava elevado em 41% e a HE4 em apenas 8%. Moore et al. (51), também estudando mulheres com massa anexial na pré-menopausa, observaram que o CA 125 estava elevado em 37% (217/593) e a HE4 elevada em apenas 6% (33/593). Assim, como tumores anexiais benignos podem ser tratados com segurança por ginecologistas gerais, a diferenciação pré-operatória das massas anexiais poderia beneficiar a assistência médica (13).

Foram encontradas diferenças significativas entre os valores médios do CA125, da HE4 e do índice ROMA em mulheres com tumores malignos, tumores benignos e mulheres saudáveis. Evidentemente, esses marcadores desempenham um papel importante na diferenciação da massa anexial, apesar de que o significado desses marcadores, com relação a mudanças na prática clínica, ainda seja o objeto de diversos estudos (54). Como método de rastreamento, nenhum marcador é recomendado em mulheres assintomáticas, sem história familiar de câncer de mama ou ovário ou portadoras de mutação do BRCA1 e BRCA2. Entretanto, atualmente, o *American College of Obstetrics and*

Gynecology recomenda que seja oferecido exame pélvico, dosagem sérica de CA125 e ultrassom transvaginal para mulheres com sintomas de câncer de ovário (55). Neste estudo, após analise multivariada, os níveis de CA125 contribuíram para diferenciação entre tumores malignos, tumores benignos e mulheres saudáveis. Dessa forma, existe uma grande expectativa de avaliar a prevalência de tumores malignos em mulheres com sintomas relacionados a câncer de ovário, de acordo com os níveis de CA125 e outros marcadores. Andersen et al. (56), no estudo prospectivo comparando 74 mulheres com câncer de ovário e 137 mulheres saudáveis, concluíram que CA125 ou HE4, quando associado ao índice de sintomas, detectavam 91,9% dos casos de neoplasia maligna. Contudo, baseado nos resultados deste estudo, não há evidência suficiente para o uso da HE4 para diferenciar mulheres com massas anexiais.

No segundo artigo deste estudo, quando se avaliou a presença de sintomas específicos relatados pelas mulheres com tumores malignos, tumores benignos e mulheres saudáveis, todas com tumores malignos relataram algum tipo de sintoma. Embora esses sintomas também fossem referidos por mulheres com tumores benignos e do grupo-controle, as frequências de dor pélvica, dor abdominal, dor nas costas, dificuldade para comer, empachamento, estômago cheio, náusea ou vômito, inchaço abdominal, aumento do volume abdominal, massa abdominal, sangramento pós-menopausa, fadiga e dificuldade para respirar, foram significativamente maiores entre as mulheres com câncer quando comparadas a mulheres com tumores benignos. Havendo vários sintomas correlatos, avaliamos os possíveis agrupamentos e identificamos pela técnica de Ward seis

agrupamentos e dois sintomas isolados: abdômen (abdômen inchado e aumento do abdômen); dor (pélvica, abdominal, costas); digestão (estômago cheio, náusea/vômito); alimentação (dificuldade de comer, empachada); diversos (cansaço, dificuldade de respirar) e urinários (urgência de urinar, urinar frequentemente); e os sintomas isolados: pernas inchadas e massa abdominal. Observamos que os agrupamentos de sintomas que se mantiveram significativamente associados com neoplasia maligna de ovário foram abdômen e digestão. A proporção de mulheres com os agrupamentos de sintomas abdômen, dor e alimentação, foi maior naquelas com tumor maligno. O agrupamento urinário foi significantemente mais prevalente nas mulheres com tumores benignos comparado com mulheres saudáveis. No geral, o agrupamento de sintomas abdômen apresentou a melhor capacidade de diferenciar mulheres com tumores malignos, tumores benignos e mulheres saudáveis. O nível de CA125, agrupamento dor e sintoma isolado - perda de peso - também contribuíram para diferenciar mulheres com tumores malignos, tumores benignos e mulheres saudáveis.

Mulheres com câncer de ovário apresentam múltiplos sintomas; entretanto, geralmente esses não são ginecológicos. Por serem essencialmente sintomas gerais, tanto as mulheres quanto os médicos tendem a subvalorizá-los. Os médicos frequentemente tratam as mulheres como portadoras de síndrome do colo irritável, estresse, gastrite ou depressão, meses antes de detectarem o câncer de ovário (7). A subvalorizarão dos sintomas pelas mulheres e pelos médicos pode contribuir para diagnósticos do câncer de ovário em estádios mais avançados. Na amostra deste estudo, todas as mulheres com tumores

malignos referiram algum tipo de sintoma, caracterizado como relevante aquele que aparecia mais de 12 vezes por mês no período menor de um ano (52). Essa amostra de mulheres brasileiras apresentou, assim, dados concordantes com outros estudos realizados em outras populações. Goff et al.(53), entrevistando 1725 mulheres com câncer de ovário dos Estados Unidos e Canadá, observaram que 95% das mulheres referiram ter tido algum sintoma três a seis meses antes de procurar o médico, independentemente do estádio. Nesse estudo, os sintomas mais comuns foram aumento do volume abdominal (77%), gastrointestinal (70%), dor (58%), constitucionais (50%), urinários (34%), e pélvicos (26%).

Os dados deste estudo demonstram que os sintomas podem ser usados até mesmo para detectar câncer de ovário em estádios iniciais. No presente estudo, 62% das mulheres com câncer tinham doença nos estádios I/II, independentemente do tipo histológico. Rossing *et al*. (57), relataram que o índice de sintomas foi positivo em 62,3% das mulheres com doenças em estádio inicial. Entretanto, as mulheres foram entrevistadas, em média, nove meses após o diagnóstico da doença. Uma limitação do presente estudo foi que não foram analisadas mulheres na pré e pós-menopausa, separadamente. Evidentemente, nestas análises, sintomas que se aplicavam somente às mulheres na pré ou pós menopausa e os sintomas aplicáveis somente a mulheres sexualmente ativas não foram incluídos no modelo multivariado. Infelizmente, esse recurso não é suficiente para descartar o viés de seleção, uma vez que, por exemplo, dor pélvica, foi significativamente associada com malignidade, e é frequentemente relatada por mulheres jovens com endometrioma (58).

Sintomas específicos, quando avaliados em relação à sua frequência e duração, foram úteis na identificação de mulheres com câncer de ovário. A presença de sintomas específicos como dos agrupamentos abdômen, dor e sintoma isolado – perda de peso –, e o nível elevado do CA125 poderão contribuir para a diferenciação pré-operatória de neoplasias malignas em mulheres com massa anexial e indicação cirúrgica. Mulheres com neoplasia maligna de ovário podem ser encaminhadas e tratadas em serviços terciários, envolvendo custos financeiros e emocionais elevados. Por sua vez, mulheres com tumores benignos podem ser conduzidas no seu local de origem.

5. Conclusões

- **Artigo 1 –** Entre as mulheres com massa anexial e nível de CA125 elevado, aquelas com nivel de HE4 elevado apresentaram probabilidade significativamente maior de ter um carcinoma de ovário. O marcador HE4 foi útil na detecção de carcinomas em estágios iniciais. No entanto, mulheres com carcinomas mucinosos e tumores borderlines de ovário, que apresentam níveis de CA125 normais, também não expressaram a HE4. A HE4 contribuiu para a identificação das mulheres com tumores benignos entre aqueles com massa anexial e nível de CA125 elevado, como, por exemplo, os endometriomas. Por outro lado, as concentrações séricas de mesotelina estiveram aumentadas em apenas 25% das mulheres com tumores malignos, essencialmente naquelas com carcinomas avançados. Com base nestes resultados, ainda não está claro se esses marcadores tumorais podem ser recomendados, na prática clínica, para a discriminação de tumores malignos em mulheres brasileiras com massas anexiais.

- **Artigo 2 –** A proporção de mulheres com tumores malignos que referiam sintomas especificos dos grupamentos: abdômen, dor, alimentação, e o sintoma perda de peso, mais que 12 vezes no mês em até um ano antes da consulta, foi maior que a de mulheres com tumores benignos, apesar desses sintomas serem relatados por mulheres com tumores benignos. O grupamento abdômen apresentou a melhor capacidade de diferenciar mulheres com tumores malignos, tumores benignos e mulheres saudáveis e o CA125, o grupamento dor e o sintoma perda de peso também contribuíram para diferenciar mulheres com tumores malignos, tumores benignos e mulheres saudáveis. Devido à raridade do câncer de ovário e à alta prevalência desses sintomas na população geral, o valor preditivo é reduzido com um método de rastreamento. Contudo, baseado nos resultados deste estudo, está claro que a avaliação de sintomas específicos pode ser recomendada na prática clínica para diferenciação pré-operatória de massas anexiais em mulheres brasileiras.

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7. Anexos

7.1. Anexo 1 – Ficha de Coleta de Dados

AVALIAÇÃO DE BIOMARCADORES PARA DETECÇÃO DE TUMORES MALIGNOS EM PACIENTES COM TUMOR ANEXIAL

I. Identificação **Ficha:** I__I__I__I

HC: I__I__I__I__I__I__I__I__I__I

Iniciais:____________

- - - - - - - - - - - ✂ -

Questionário

Ficha: I__I__I__I

1. Qual a data do seu nascimento ? I__I__I / I__I__I /I__I__I

2. Peso? I__I__I__IKg

3. Altura? I__I__I__Im

4.As perguntas a seguir pedem sua opinião sobre sua saúde com um todo. Se você teve algum dos seguintes sintomas no ano passado. Se eles estavam presentes em que freqüência (número de dias no mês) e qual a duração (há quanto tempo).

a. Sente dor pelvica (dor na parte abaixo da barriga)? I__I sim I__I não **passe para b**

a.1. Qual a intensidade da dor?
I__I mínima I__I fraca I__I forte I__I fortíssima

a.2. Quantos dias por mês dura essa dor?
I__I < 1 I__I 1-2 I__I 3-6 I__I 7-12 I__I 13-19 I__I >20

a.3. Há quanto tempo essa dor persiste (meses)?
I__I < 1 I__I 1-2 I__I 3-4 I__I 5-6 I__I 7-9 I__I 10-12 I__I >12

b. Sente dor abdominal? |__| sim |__| não **passe para c**

b.1. Qual a intensidade da dor?
|__| mínima |__| fraca |__| forte |__| fortíssima

b.2. Quantos dias por mês dura essa dor?
|__| < 1 |__| 1-2 |__| 3-6 |__| 7-12 |__| 13-19 |__| >20

b.3. Há quanto tempo essa dor persiste (meses)?
|__| < 1 |__| 1-2 |__| 3-4 |__| 5-6 |__| 7-9 |__| 10-12 |__| >12

c. Sente dor nas costas? |__| sim |__| não **passe para d**

c.1. Qual a intensidade da dor?
|__| mínima |__| fraca |__| forte |__| fortíssima

c.2. Quantos dias por mês dura essa dor?
|__| < 1 |__| 1-2 |__| 3-6 |__| 7-12 |__| 13-19 |__| >20

c.3. Há quanto tempo essa dor persiste (meses)?
|__| < 1 |__| 1-2 |__| 3-4 |__| 5-6 |__| 7-9 |__| 10-12 |__| >12

d. Tem dificuldade para comer normalmente? |__| sim |__| não **passe para e**

d.1. Qual a intensidade da dificuldade?
|__| mínima |__| pouca |__| bastante |__| grande

d.2. Quantos dias por mês dura essa dificuldade?
|__| < 1 |__| 1-2 |__| 3-6 |__| 7-12 |__| 13-19 |__| >20

d.3. Há quanto tempo essa dificuldade persiste (meses)?
|__| < 1 |__| 1-2 |__| 3-4 |__| 5-6 |__| 7-9 |__| 10-12 |__| >12

e. Sente-se empachada? |__| sim |__| não **passe para f**

e.1. Qual a intensidade desse sintoma?
|__| mínima |__| fraca |__| forte |__| fortíssima

e.2. Quantos dias por mês dura esse sintoma?
|__| < 1 |__| 1-2 |__| 3-6 |__| 7-12 |__| 13-19 |__| >20

e.3. Há quanto tempo esse sintoma persiste (meses)?
|__| < 1 |__| 1-2 |__| 3-4 |__| 5-6 |__| 7-9 |__| 10-12 |__| >12

f. Sente o estômago cheio rapidamente? |__| sim |__| não **passe para g**

f.1. Qual a intensidade da sensação?
|__| mínima |__| fraca |__| forte |__| fortíssima

f.2. Quantos dias por mês dura essa sensação?
|__| < 1 |__| 1-2 |__| 3-6 |__| 7-12 |__| 13-19 |__| >20

f.3. Há quanto tempo essa sensação persiste (meses)?
|__| < 1 |__| 1-2 |__| 3-4 |__| 5-6 |__| 7-9 |__| 10-12 |__| >12

g.Tem sentido náusea ou vomitado? I__I sim I__I não **passe para i**

g.1. Qual a intensidade desse sintoma?
I__I mínima I__I fraca I__I forte I__I fortíssima

g.2. Quantos dias por mês dura esse sintoma?
I__I < 1 I__I 1-2 I__I 3-6 I__I 7-12 I__I 13-19 I__I >20

g.3. Há quanto tempo esse sintoma persiste (meses)?
I__I < 1 I__I 1-2 I__I 3-4 I__I 5-6 I__I 7-9 I__I 10-12 I__I >12

h. Perdeu peso? I__I sim I__I não **passe para i**

h.1. Qual a intensidade dessa perda?
I__I mínima I__I fraca I__I forte I__I fortíssima

h.2. Há quanto tempo essa perda de peso persiste (meses)?
I__I < 1 I__I 1-2 I__I 3-4 I__I 5-6 I__I 7-9 I__I 10-12 I__I >12

i. Sente o abdome inchado? I__I sim I__I não **passe para j**

i.1. Qual a intensidade desse sintoma?
I__I mínima I__I fraca I__I forte I__I fortíssima

i.2. Quantos dias por mês dura esse sintoma?
I__I < 1 I__I 1-2 I__I 3-6 I__I 7-12 I__I 13-19 I__I >20

i.3. Há quanto tempo esse sintoma persiste (meses)?
I__I < 1 I__I 1-2 I__I 3-4 I__I 5-6 I__I 7-9 I__I 10-12 I__I >12

j. Aumento do volume abdominal? I__I sim I__I não **passe para k**

j.1. Qual a intensidade desse sintoma?
I__I mínima I__I fraca I__I forte I__I fortíssima

j.2. Quantos dias por mês dura esse sintoma?
I__I < 1 I__I 1-2 I__I 3-6 I__I 7-12 I__I 13-19 I__I >20

j.3. Há quanto tempo esse sintoma persiste (meses)?
I__I < 1 I__I 1-2 I__I 3-4 I__I 5-6 I__I 7-9 I__I 10-12 I__I >12

k. Sente alguma massa abdominal? I__I sim I__I não **passe para l**

k.1. Qual a intensidade desse sintoma?
I__I mínima I__I fraca I__I forte I__I fortíssima

k.2. Quantos dias por mês dura essa massa?
I__I < 1 I__I 1-2 I__I 3-6 I__I 7-12 I__I 13-19 I__I >20

k.3. Há quanto tempo essa massa persiste (meses)?
I__I < 1 I__I 1-2 I__I 3-4 I__I 5-6 I__I 7-9 I__I 10-12 I__I >12

l. Tem urgência de urinar? l__l sim l__l não **passe para m**

l.1. Qual a intensidade desse sintoma?
l__l mínima l__l fraca l__l forte l__l fortíssima

l.2. Quantos dias por mês dura esse sintoma?
l__l < 1 l__l 1-2 l__l 3-6 l__l 7-12 l__l 13-19 l__l >20

l.3. Há quanto tempo esse sintoma persiste (meses)?
l__l < 1 l__l 1-2 l__l 3-4 l__l 5-6 l__l 7-9 l__l 10-12 l__l >12

m. Tem necessidade de urinar frequentemente? l__l sim l__l não **passe para n**

m.1. Qual a intensidade desse sintoma?
l__l mínima l__l fraca l__l forte l__l fortíssima

m.2. Quantos dias por mês dura esse sintoma?
l__l < 1 l__l 1-2 l__l 3-6 l__l 7-12 l__l 13-19 l__l >20

m.3. Há quanto tempo essa dor persiste (meses)?
l__ l < 1 l__ l 1-2 l__ l 3-4 l__ l 5-6 l__ l 7-9 l__ l 10-12 l__ l >12

n. Tem prisão de ventre? l__l sim l__l não **passe para o**

n.1. Qual a intensidade desse sintoma?
l__l mínima l__l fraca l__l forte l__l fortíssima

n.2. Quantos dias por mês dura esse sintoma?
l__l < 1 l__l 1-2 l__l 3-6 l__l 7-12 l__l 13-19 l__l >20

n.3. Há quanto tempo esse sintoma persiste (meses)?
l__l < 1 l__l 1-2 l__l 3-4 l__l 5-6 l__l 7-9 l__l 10-12 l__l >12

o. Tem diarréia? l__l sim l__l não **passe para p**

o.1. Qual a intensidade desse sintoma?
l__l mínima l__l fraca l__l forte l__l fortíssima

o.2. Quantos dias por mês dura esse sintoma?
l__l < 1 l__l 1-2 l__l 3-6 l__l 7-12 l__l 13-19 l__l >20

o.3. Há quanto tempo esse sintoma persiste (meses)?
l__l < 1 l__l 1-2 l__l 3-4 l__l 5-6 l__l 7-9 l__l 10-12 l__l >12

p. Qual foi a data da sua última menstruação? Data: l__l__/__l__/__l__l
menacme l__l menopausada l__l passe para q

p.1. O ciclo menstrual é regular? l__ lsim **passe para r** l__lnão

p.2. Qual a freqüência da irregularidade?
l__l mínima l__l fraca l__l forte l__l fortíssima

p.3. Quanto tempo (meses) dura essa irreguladade ?
|__| < 1 |__| 1-2 |__| 3-6 |__| 7-12 |__| 13-19 |__| >20

p.4. Há quanto tempo essa irregularidade persiste (meses)?
|__| < 1 |__| 1-2 |__| 3-4 |__| 5-6 |__| 7-9 |__| 10-12 |__| >12

q. Tem sangramento após a menopausa? |__| sim |__| não **passe para r**

q.1. Qual a intensidade do sangramento?
|__| mínima |__| fraca |__| forte |__| fortíssima

q.2. Quantos dias por mês dura esse sangramento?
|__| < 1 |__| 1-2 |__| 3-6 |__| 7-12 |__| 13-19 |__| >20

q.3. Há quanto tempo esse sangramento persiste (meses)?
|__| < 1 |__| 1-2 |__| 3-4 |__| 5-6 |__| 7-9 |__| 10-12 |__| >12

r. Tem dor durante a relação sexual? |__| sim |__| não **passe para s**

r.1. Qual a intensidade da dor?
|__| mínima |__| fraca |__| forte |__| fortíssima

r.2. Quantos dias por mês dura essa dor?
|__| < 1 |__| 1-2 |__| 3-6 |__| 7-12 |__| 13-19 |__| >20

r.3. Há quanto tempo essa dor persiste (meses)?
|__| < 1 |__| 1-2 |__| 3-4 |__| 5-6 |__| 7-9 |__| 10-12 |__| >12

s. Tem sangramento com a relação sexual? |__| sim |__| não **passe para t**

s.1. Qual a intensidade desse sangramento?
|__| mínima |__| fraca |__| forte |__| fortíssima

s.2. Quantos dias por mês dura esse sangramento?
|__| < 1 |__| 1-2 |__| 3-6 |__| 7-12 |__| 13-19 |__| >20

s.3. Há quanto tempo esse sangramento persiste (meses)?
|__| < 1 |__| 1-2 |__| 3-4 |__| 5-6 |__| 7-9 |__| 10-12 |__| >12

t. Sente cansaço(fadiga)? |__| sim |__| não **passe para u**

t.1. Qual a intensidade desse cansaço?
|__| mínima |__| fraca |__| forte |__| fortíssima

t.2. Quantos dias por mês dura esse cansaço?
|__| < 1 |__| 1-2 |__| 3-6 |__| 7-12 |__| 13-19 |__| >20

t.3. Há quanto tempo esse cansaço persiste (meses)?
|__| < 1 |__| 1-2 |__| 3-4 |__| 5-6 |__| 7-9 |__| 10-12 |__| >12

u. Sente as pernas inchadas? I__I sim I__I não **passe para v**

u.1. Qual a intensidade desse sintoma?
I__I mínima I__I fraca I__I forte I__I fortíssima

u.2. Quantos dias por mês dura esse sintoma?
I__I < 1 I__I 1-2 I__I 3-6 I__I 7-12 I__I 13-19 I__I >20

u.3. Há quanto tempo esse sintoma persiste (meses)?
I__I < 1 I__I 1-2 I__I 3-4 I__I 5-6 I__I 7-9 I__I 10-12 I__I >12

v. Tem dificuldade para respirar? I__I sim I__I não **passe para x**

v.1. Qual a intensidade desse sintoma?
I__I mínima I__I fraca I__I forte I__I fortíssima

v.2. Quantos dias por mês dura esse sintoma?
I__I < 1 I__I 1-2 I__I 3-6 I__I 7-12 I__I 13-19 I__I >20

v.3. Há quanto tempo esse sintoma persiste (meses)?
I__I < 1 I__I 1-2 I__I 3-4 I__I 5-6 I__I 7-9 I__I 10-12 I__I >12

x. outro sintoma? I__I sim **Qual** : _____
I__I não **passe para y**

x.1. Qual a intensidade desse sintoma?
I__I mínima I__I fraca I__I forte I__I fortíssima

x.2. Quantos dias por mês dura esse sintoma?
I__I < 1 I__I 1-2 I__I 3-6 I__I 7-12 I__I 13-19 I__I >20

x.3. Há quanto tempo esse sintoma persiste (meses)?
I__I < 1 I__I 1-2 I__I 3-4 I__I 5-6 I__I 7-9 I__I 10-12 I__I >12

y. Nenhum sintoma I__I sim

Ficha

1. Anátomo-patológico: Data: I__I__/__I__/__I__I Nº I__I__I__I__I__I__I NR I_I
Resultado:_____

2. Biomarcadores **Número da amostra**
I__I__I__I

2.1. CA 125 I__I__I__I__IU/ml Data: I__I__/__I__/__I__I

2.2. Mesotelina I__I__I__I__InM Data: I__I__/__I__/__I__I

2.3. HE4 I__I__I__I__IpM Data: I__I__/__I__/__I__I

7.2. Anexo 2 – Termo de Consentimento Livre e Esclarecido

Obs: termo de consentimento comum a três projetos de pesquisa relacionados a mesma paciente.

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO PARA PACIENTES

Avaliação de biomarcadores para detecção de tumores malignos em pacientes com tumor anexial. Pesquisadora Responsável: Denise da Rocha Pitta Lima de Moraes - número telefone (19) 3521-9423

Achados Clínicos, ultrassonográficos e bioquímicos como preditores de malignidade em mulheres com tumores anexiais. Pesquisador Responsável: Caio Augusto Hartman - número telefone (19) 3521-9305

Laparoscopia na abordagem inicial de tumores ovarianos. Pesquisador Responsável: Amílcar Barreta – número telefone: (19) 3521905

Eu, Sra_____, atendida no ambulatório de oncologia pélvica – ovário do Centro de Atenção Integral a Saúde da Mulher (CAISM)-UNICAMP fui convidada a participar destas pesquisas por apresentar tumor na pelve, dentro da barriga e tendo necessidade de cirurgia para saber se o tumor é maligno ou benigno e para realizar o tratamento da minha doença. Essas pesquisas têm como objetivo verificar se há melhora na capacidade de classificar tumores de ovário em benignos e malignos (câncer) antes da cirurgia. Para isso avaliaremos: 1) a dosagem no sangue das substâncias chamadas de marcadores tumorais, CA 125, mesotelina, HE4; 2) o exame de ultrassonografia; 3) a avaliação de vários sintomas e; 4) o índice de risco de malignidade; separados e em conjunto.

Os critérios clínicos encontrados durante a consulta associados aos resultados dos exames de ultrassonografia e CA 125 serão utilizados para definição e indicação médica do tipo de tratamento cirúrgico a ser realizado que poderá consistir em: videolaparoscopia (técnica cirúrgica pouco invasiva, que consiste na realização de 3 ou 4 pequenos cortes) ou laparotomia (técnica cirúrgica, que consiste na realização de um corte maior na barriga). A pesquisa não mudara em nada o tratamento que seria feito com você se você não participasse da pesquisa.

Sei que responderei a um questionário, com duração prevista de 20 a 30 minutos, com perguntas sobre informações pessoais. Essas perguntas serão feitas pelos responsáveis pela pesquisa, em uma única entrevista, antes da consulta médica, em uma sala do ambulatório de oncologia pélvica, não atrapalhando o meu atendimento. As fichas ficarão de posse do responsável pela pesquisa, que manterá o sigilo da fonte destas informações, mantendo o meu anonimato.

Sei que para este estudo será realizada a coleta de uma amostra de sangue por punção venosa, semelhante a uma injeção na veia sendo aspirada pequena quantidade de sangue que ficará armazenada no Laboratório Clinico Especializado para quantificação das substâncias chamadas de marcadores tumorais: CA125, mesotelina e HE4. O exame de ultrassonografia será agendado após a consulta no ambulatório de ovário e será feito no setor de ultrassonografia do CAISM que fica no andar térreo. Esse exame não dói nem faz mal à saúde e é realizado passando-se gel e aplicando-se um aparelho sobre a pele do abdome, ou utilizando-se um aparelho que é colocado no canal vaginal (ultrassom transvaginal), protegido por um condom (camisinha), a critério do médico que realizará o exame. Para realizar este exame a Sra permanecerá deitada por cerca de 20 minutos.

É necessária realização de cirurgia para diagnóstico definitivo, tratamento e estadiamento do meu tumor que poderá ser realizada de uma das seguintes formas: 1) videolaparoscopia ou; 2) laparotomia. Estas técnicas de cirurgia não são novas nem tampouco experimentais, existem há vários anos e suas técnicas e usos estão consolidadas. Sua indicação será baseada em critérios clínicos bem estabelecidos. A cirurgia por videolaparoscopia consiste em 3 ou 4 pequenos cortes na barriga de tamanho necessário à introdução de instrumental cirúrgico dentro da barriga o qual é usado para o tratamento do tumor tratando-se de técnica considerada pouco invasiva com vantagens bem estabelecidas na recuperação das pacientes após a cirurgia e na redução do tempo de retorno às atividades habituais, porém é técnica mais complexa , dependente de material especializado, e de realização dificultada por fatores como tumores de grande tamanho, aderências e outros. A laparotomia consiste em cirurgia com corte extenso em pé, no meio da barriga para permitir o acesso ao tumor localizado dentro da barriga, possui vantagens ao permitir manipulação mais fácil do tumor e não ser dependente de material especializado, porém está associada a mais dor no pós-operatório e recuperação mais lenta e maior demora no retorno às atividades habituais. Sempre que se propõe cirurgia por videolaparoscopia pode haver a necessidade de conversão para laparotomia a depender de necessidade e avaliação do cirurgião no momento da realização da cirurgia.

Só participarei da pesquisa intitulada “Laparoscopia na Avaliação Inicial de Tumores Ovarianos” caso o médico que me atender, baseando-se em critérios clínicos e no resultado de meus exames, indique a realização de cirurgia por videolaparoscopia.

Fui esclarecida que a participação nestas pesquisas é totalmente voluntária. Sei que não serei paga para participar destes estudos. **A não aceitação na participação nas pesquisas não implicará na perda dos direitos iniciais rotineiramente oferecidos pelo hospital.** Aceitando participar, não terei privilégios adicionais no atendimento.

Os possíveis benefícios que essas pesquisas possam trazer ao tratamento de pacientes com tumores ovarianos, só poderão ser utilizados após o término das mesmas, portanto, estas pesquisas não trarão nenhum privilégio ou benefício imediato. Também não acarretarão prejuízos.

Autorizo os responsáveis pelas pesquisas a examinarem meus registros médicos a fim de verificar informações relacionadas aos objetivos das pesquisas, para que sejam anexados às fichas de pesquisa. No entanto, os registros médicos serão tratados confidencial e sigilosamente.

Tenho o direito de fazer perguntas para esclarecer minhas dúvidas sobre minha participação em qualquer momento da entrevista, podendo desistir de participar desta pesquisa a qualquer momento, mesmo após a realização dos exames, sem nenhum prejuízo ou alteração no meu tratamento.

Em caso de dúvidas ou esclarecimento, tenho o direito de telefonar para os pesquisadores responsáveis, para a Dra Sophie Derchain, Profa Dra Pesquisadora orientadora dos projetos no número (19) 3521-9305 ou para o Comitê de Ética em Pesquisa da FCM/UNICAMP no número (19) 3521-8936.

Paciente_____

RG_____

Pesquisadores_____

Campinas,_____**de**_____**de 2009/10/11/12**

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO PARA CONTROLES

Avaliação de biomarcadores para detecção de tumores malignos em pacientes com tumor anexial. Pesquisadora Responsável: Denise da Rocha Pitta Lima de Moraes - número telefone (19) 3521-9423

Eu,Sra_____, atendida nos ambulatórios da UNICAMP fui convidada a participar desta pesquisa que tem como objetivo comparar a dosagem no sangue das substâncias chamadas de marcadores tumorais, CA 125, mesotelina, HE4 e a avaliação de vários sintomas de voluntárias saudáveis, que não possuam qualquer diagnóstico de doença ovariana com as dosagens no sangue e os vários sintomas de mulheres com tumor anexial para ver se há melhora na capacidade de classificar tumores de ovário em benignos e malignos (câncer) antes da cirurgia.

Sei que responderei a um questionário, com duração prevista de 20 a 30 minutos, com perguntas sobre informações pessoais. Essas perguntas serão feitas pela pesquisadora responsável pela pesquisa, Bióloga Denise Pitta, em uma única entrevista, nos ambulatórios, após consulta médica e no posto de coleta, antes de coleta de material biológico, não atrapalhando o meu atendimento. As fichas ficarão de posse do responsável pela pesquisa, que manterá o sigilo da fonte destas informações, mantendo o meu anonimato.

Sei que para este estudo será realizada a coleta de uma amostra de sangue por punção venosa, semelhante a uma injeção na veia sendo aspirada pequena quantidade de sangue que ficará armazenada no Laboratório Clinico Especializado para quantificação das substâncias chamadas de marcadores tumorais: CA125, mesotelina e HE4.

Fui esclarecida que a participação nesta pesquisa é totalmente voluntária. Sei que não serei paga para participar deste estudo. **A não aceitação na participação no estudo não implicará na perda dos direitos iniciais rotineiramente oferecidos pelo hospital.** Aceitando participar, não terei privilégios adicionais no atendimento.

Esta pesquisa não trará nenhum benefício imediato. Também não acarretará prejuízos. Os possíveis benefícios que essa pesquisa possa trazer ao tratamento de pacientes com tumores ovarianos, só poderão ser utilizados após a pesquisa terminar.

Autorizo a Bióloga Denise Pitta examinar meus registros médicos a fim de verificar informações relacionadas ao objetivo da pesquisa, para que sejam anexados às fichas de pesquisa. No entanto, os registros médicos serão tratados confidencialmente.

Tenho o direito de fazer perguntas para esclarecer minhas dúvidas sobre minha participação em qualquer momento da entrevista, podendo desistir de participar durante ou no final da entrevista.

Em caso de dúvidas ou esclarecimento, tenho o direito de telefonar para a Dra Sophie Derchain, Profa Dra Pesquisadora orientadora do projeto e para Bióloga Denise Pitta no número (19) 3521-9305 ou para o Comitê de Ética em Pesquisa da FCM/UNICAMP no número (19) 3521-8936.

Paciente_____

Campinas,_____de_____de 2009/10/11/12

Pesquisador_____

Campinas,_____de_____de 2009/10/11/12

7.3. Anexo 3 – Parecer do CEP

FACULDADE DE CIÊNCIAS MÉDICAS
COMITÊ DE ÉTICA EM PESQUISA

www.fcm.unicamp.br/pesquisa/etica/index.html

CEP, 24/11/09.
(Grupo III)

PARECER CEP: N° 1092/2009 (Este n° deve ser citado nas correspondências referente a este projeto)
CAAE: 0849.0.146.000-09

I - IDENTIFICAÇÃO:

PROJETO: **"AVALIAÇÃO DE BIOMARCADORES PARA DETECÇÃO DE TUMORES MALIGNOS EM MULHERES COM TUMOR ANEXIAL".**
PESQUISADOR RESPONSÁVEL: Denise da Rocha Pitta Lima de Moraes/Sophie Françoise Mauricette Derchain
INSTITUIÇÃO: CAISM/UNICAMP
APRESENTAÇÃO AO CEP: 11/11/2009
APRESENTAR RELATÓRIO EM: 24/11/10 (O formulário encontra-se no *site* acima)

II - OBJETIVOS

Avaliar a sensibilidade e especificidade do CA 125, HE4, mesotelina no diagnóstico de tumores malignos de ovário em mulheres submetidas a laparotomia por massa anexial associada aos sintomas e fatores pessoais.

III - SUMÁRIO

Trata-se de um estudo clínico prospectivo para o qual serão selecionados 93 casos malignos, 149 casos benignos e 149 casos sem a doença, totalizando uma amostra de 391casos. O sangue periférico será coletado em tubo seco de 10ml de maneira a termos amostra suficiente para quantificação dos três biomarcadores através de imunoensaio enzimático. Os dados serão tabulados em planilhas eletrônicas no programa Excel. A distribuição das variáveis de controle nos grupos de estudo será avaliada através de regressão logística. Serão calculadas a sensibilidade e especificidade, bem como seus intervalos de confiança em 95% (IC95%) para cada um dos biomarcadores (e a combinação destes) na detecção dos tumores ovarianos em diferentes pontos de corte. Será usada a curva ROC (Receiver Operator Characterist) para a determinação dos melhores pontos de corte. Será comparada as áreas sob as curvas ROC para cada marcador através do método não paramétrico de De Long.

IV - COMENTÁRIOS DOS RELATORES

O projeto apresenta-se bem redigido, com metodologia adequada. Os critérios de inclusão, exclusão e descontinuação dos sujeitos estão bem definidos; cálculo do tamanho amostral e análise estatistica muito bem embasados por cálculos estatísticos. Os aspectos éticos estão bem discutidos no corpo do projeto e o Termo de Consentimento Livre e Esclarecido é claro e adequado às recomendações. O orçamento é detalhado e prevê encaminhamento para agência de fomento.

Comitê de Ética em Pesquisa - UNICAMP
Rua: Tessália Vieira de Camargo, 126
Caixa Postal 6111
13083-887 Campinas – SP

FONE (019) 3521-8936
FAX (019) 3521-7187
cep@fcm.unicamp.br

V - PARECER DO CEP

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP, após acatar os pareceres dos membros-relatores previamente designados para o presente caso e atendendo todos os dispositivos das Resoluções 196/96 e complementares, resolve aprovar sem restrições o Protocolo de Pesquisa, o Termo do Consentimento Livre e Esclarecido, bem como todos os anexos incluídos na pesquisa supracitada.

O conteúdo e as conclusões aqui apresentados são de responsabilidade exclusiva do CEP/FCM/UNICAMP e não representam a opinião da Universidade Estadual de Campinas nem a comprometem.

VI - INFORMAÇÕES COMPLEMENTARES

O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (Res. CNS 196/96 – Item IV.1.f) e deve receber uma cópia do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (Item IV.2.d).

Pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou (Res. CNS Item III.1.z), exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade do regime oferecido a um dos grupos de pesquisa (Item V.3.).

O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Res. CNS Item V.4.). É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Em caso de projeto do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial (Res. 251/97, Item III.2.e)

Relatórios parciais e final devem ser apresentados ao CEP, de acordo com os prazos estabelecidos na Resolução CNS-MS 196/96.

VII – DATA DA REUNIÃO

Homologado na XI Reunião Ordinária do CEP/FCM, em 24 de novembro de 2009.

Profa. Dra. Carmen Silvia Bertuzzo
VICE-PRESIDENTE do COMITÊ DE ÉTICA EM PESQUISA
FCM / UNICAMP

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