Cancer Screening: A Mathematical Model Relating Secreted Blood Biomarker Levels to Tumor Sizes

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Abbreviations: PSA, prostate-specific antigen

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ABSTRACT

Background

Increasing efforts and financial resources are being invested in early cancer detection research. Blood assays detecting tumor biomarkers promise noninvasive and financially reasonable screening for early cancer with high potential of positive impact on patients' survival and quality of life. For novel tumor biomarkers, the actual tumor detection limits are usually unknown and there have been no studies exploring the tumor burden detection limits of blood tumor biomarkers using mathematical models. Therefore, the purpose of this study was to develop a mathematical model relating blood biomarker levels to tumor burden.

Methods and Findings

Using a linear one-compartment model, the steady state between tumor biomarker secretion into and removal out of the intravascular space was calculated. Two conditions were assumed: (1) the compartment (plasma) is well-mixed and kinetically homogenous; (2) the tumor biomarker consists of a protein that is secreted by tumor cells into the extracellular fluid compartment, and a certain percentage of the secreted protein enters the intravascular space at a continuous rate. The model was applied to two pathophysiologic conditions: tumor biomarker is secreted (1) exclusively by the tumor cells or (2) by both tumor cells and healthy normal cells. To test the model, a sensitivity analysis was performed assuming variable conditions of the model parameters. The model parameters were primed on the basis of literature data for two established and well-studied tumor biomarkers (CA125 and prostate-specific antigen [PSA]). Assuming biomarker secretion by tumor cells only and 10% of the secreted tumor biomarker reaching the plasma, the calculated minimally detectable tumor sizes ranged between 0.11 mm^3 and 3,610.14 mm^3 for CA125 and between 0.21 mm^3 and 131.51 mm^3 for PSA. When biomarker secretion by healthy cells and tumor cells was assumed, the calculated tumor sizes leading to positive test results ranged between 116.7 mm^3 and 1.52 $\times 10^6$ mm^3 for CA125 and between 27 mm^3 and 3.45 $\times 10^5$ mm^3 for PSA. One of the limitations of the study is the absence of quantitative data available in the literature on the secreted tumor biomarker amount per cancer cell in intact whole body animal tumor models or in cancer patients. Additionally, the fraction of secreted tumor biomarkers actually reaching the plasma is unknown. Therefore, we used data from published cell culture experiments to estimate tumor cell biomarker secretion rates and assumed a wide range of secretion rates to account for their potential changes due to field effects of the tumor environment.

Conclusions

This study introduced a linear one-compartment mathematical model that allows estimation of minimal detectable tumor sizes based on blood tumor biomarker assays. Assuming physiological data on CA125 and PSA from the literature, the model predicted detection limits of tumors that were in qualitative agreement with the actual clinical performance of both biomarkers. The model may be helpful in future estimation of minimal detectable tumor sizes for novel proteomic biomarker assays if sufficient physiologic data for the biomarker are available. The model may address the potential and limitations of tumor biomarkers, help prioritize biomarkers, and guide investments into early cancer detection research efforts.

The Editors' Summary of this article follows the references.

Introduction

Many cancer types are likely curable by conventional therapies, if detected early enough. Therefore, early detection is a primary objective of cancer research with a high potential of improving both patients' survival and quality of life. To reach the major goal of these efforts—detection of cancer in early stages when the disease may still be curable—currently two types of cancer early detection tests are most widely studied: blood test(s) and imaging. In both diagnostic fields, tremendous progress has been made during the last decade. Ideally, a less costly highly accurate blood-based diagnostic biomarker test would precede any further imaging or biopsy studies. With emerging new, highly sensitive proteomics test assays there seems to be a nearly limitless potential to detect traces of tumor biomarkers in patient serum, if in fact they exist and are specific enough at early tumor stages [1–3]. The advances in these test assays pose the question: What is a realistic lower detectable tumor size limit for these diagnostic tests? To answer this question for secreted blood biomarkers, a mathematical compartmental model simulating the kinetics of blood biomarkers under varying physiological conditions can be utilized. Many aspects have to be taken into consideration when a model of a new test assay is being developed. We chose the setting of ovarian cancer and prostate cancer to test our model, because they are among the cancer types that meet the profile for early detection. Both cancer types often remain relatively asymptomatic until they are advanced and there is likely significant opportunity to improve survival through early detection of the disease.

Patients diagnosed with early stages of ovarian cancer when the disease is confined to the ovary have a 5-y survival of up to 95% following conventional therapy. In late stages, however, the 5-y survival is 25%–30%. Unfortunately, the vast majority of patients (70%) are still diagnosed in these advanced stages [4,5]. For ovarian cancer, the tumor biomarker CA125 that is established for the post-treatment follow up of ovarian cancer patients has been extensively explored, and data on mean serum levels in healthy and patient populations, tumor cell secretion rate, and plasma half-life are available in the literature [6–8]. Adequate diagnostic tools for the early detection of ovarian cancer are still missing, but extensive research in the field of novel serum tumor biomarkers and diagnostic imaging is underway [1,9–13]. Prostate cancer has a high prevalence in detectable preclinical stages. The introduction of the blood biomarker prostate-specific antigen (PSA) for screening of prostate cancer has already caused a "stage migration" [14]. The introduction of screening for prostate cancer using PSA and digital rectal examination has resulted in increased detection of prostate cancer in early stages when the disease is still curable. However, it remains controversial whether screening for prostate cancer using this approach has yet had substantial impact on the overall survival of men with prostate cancer [15]. A high percentage of men diagnosed with prostate cancer through screening would not develop symptoms or die of the disease even if left untreated [16]. On the other hand, aggressive treatment of those more indolent prostate cancer cases may have harmful effects and cause significant morbidity [17]. Nevertheless, some authors conclude that men in the general population

without increased risk for prostate cancer in the age range of 50–70 y may benefit from screening with PSA [16].

To our knowledge, there have been no studies exploring the tumor burden detection limits of serum tumor biomarkers using mathematical models. Because of increasing research into early detection of cancer, especially in the screening use of blood biomarkers, studies using mathematical models could have an important impact on cancer detection research [1,18]. Such mathematical models may address the potential and priority of tumor biomarkers, and guide investments into research efforts. Moreover, the actual protein detection limits that are required in order to improve disease outcome are usually unknown for the emerging blood biomarkers.

Therefore, the purpose of this study was to develop a mathematical model to describe the potential detection limits of tumor burden based on blood biomarkers. To test the model, a sensitivity analysis was performed using literature data on two established blood tumor biomarkers for which relevant model parameter data are available, CA125 and PSA.

Methods

For the compartmental model, the following conditions were assumed: (1) the compartment represents the plasma, which is considered well mixed and kinetically homogenous; (2) the tumor biomarker of interest consists of a protein that is secreted by tumor cells into the extracellular fluid compartment, and that a certain percentage of the secreted protein will enter the intravascular space (plasma) at a continuous rate. In the plasma, the protein of interest has a distinct half-life owing to, e.g., degradation by proteases, hepatic metabolism, or in the case of a smaller protein because of filtration by the kidney. Two potential pathophysiologic conditions were assumed: (1) the tumor biomarker is either secreted by the tumor cells only (no background secretion by normal cells) or (2) by tumor cells and to some extent by healthy normal cells (background secretion by healthy cells).

The balance (steady state) between protein secretion into (inflow) and protein removal out of the intravascular space (outflow) was calculated using a linear one-compartment model (Figure 1) [19].

In steady state (no change in biomarker plasma/serum level with respect to time), the plasma/serum level of the tumor biomarker can be calculated using formula c as a function of the influx of tumor biomarker via secretion by normal and tumor cells into the plasma corrected for the constant elimination (efflux) of the tumor biomarker out of the plasma/serum (shown in Figure 1). Alternatively, this formula can also be used to calculate the secretion rate of the tumor biomarker, if the amount of tumor biomarker in plasma/serum and the biomarker half-life in plasma/serum is known. If the tumor biomarker is secreted by tumor cells only (meaning no secretion by healthy cells is present), the cut-off level between normal and pathologic tumor biomarker plasma/serum amounts is basically defined by the detection limit or sensitivity of the applied protein assay. The more sensitive the applied protein assay is; the lower will be the cut-off level for differentiation between healthy and pathologic tumor biomarker levels. However, if there is tumor biomarker secretion by healthy cells in addition to tumor
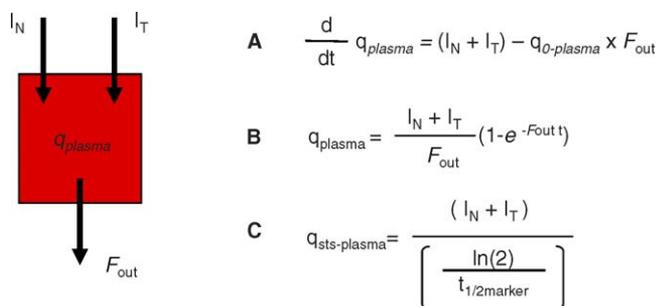
Figure 1. One-Compartment Model

The kinetics of the serum tumor biomarker in a one-compartment model can be described with formula a (d/dt reflects the derivative with respect to time) as a function of tumor biomarker influx via secretion by normal and tumor cells minus tumor biomarker level at time point t_0 times the elimination rate (efflux) of the biomarker from the intravascular compartment.

The tumor biomarker concentration in the patient plasma at a given time point can be described with formula b as a function of tumor biomarker influx via secretion by normal and tumor cells divided by the elimination of the biomarker out of the intravascular space corrected for the changes during the elapsed time.

When time is set to infinity, the steady state of the plasma biomarker concentration (no change with respect to time) can be calculated as shown in formula c: the influx of tumor biomarker via secretion by normal and tumor cells into the plasma corrected for the constant efflux of the tumor biomarker out of the plasma/serum.

Note: e, Euler's number ($\sim$2.718); F_{out}, efflux of tumor biomarker via elimination from the intravascular compartment over time (U/ml/h or ng/ml/h) (due to degradation/removal); I_N, influx of biomarker from normal healthy cells (U/ml/h or ng/ml/h); I_T, inflow of biomarker from tumor cells (U/ml/h or ng/ml/h); ln, natural logarithm; $q_{0-plasma}$, tumor biomarker plasma level at time point t_0 (U/ml or ng/ml); q_{plasma}, tumor biomarker plasma level (U/ml or ng/ml); $q_{sts-plasma}$, tumor biomarker level at steady state (U/ml or ng/ml).

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cells, the cut-off level for the distinction between normal and pathologic tumor biomarker amounts in serum is substantially higher. Here, a safety margin will likely prevent most false-positive results. In this case, by subtracting the mean biomarker level in healthy individuals (steady state reached by secretion from normal cells) from the biomarker cut-off level that is defined for differentiation between healthy and disease (steady state reached by secretion from normal and tumor cells), the tumor-secreted fraction of the biomarker level can be derived (steady state reached by secretion from tumor cells). The tumor-secreted fraction of the biomarker can then be used to calculate the secretion rate of the biomarker by tumor cells using formula c (Figure 1). The calculated secretion rate will represent the tumor biomarker secretion rate per milliliter serum. However, in order to know the total extent of tumor biomarker secretion by all tumor cells, this value has to be extrapolated to the total serum/plasma volume of the patient. When the extrapolated total amount of biomarker in the total body plasma/serum volume is known and the tumor biomarker secretion rate by tumor cells and the percentage of tumor biomarker reaching the intravascular space are defined, the required minimal number of tumor cells secreting the tumor biomarker can be calculated. Once the minimal tumor cell number is known, the required minimal tumor size leading to pathologic tumor biomarker levels in the serum can be estimated [20].

Thus, a model describing the kinetics of serum tumor biomarker secretion by tumor cells and by normal cells may

Table 1. General Parameters of the Tumor Biomarker Model CA125

Parameter	Value	Reference
Mean plasma/serum volume in a 70-kg female patient	3,150 ml	[31]
Serum half-life of serum tumor biomarker CA125	151.2 h	[7]
Mean serum level of tumor biomarker (CA125) in healthy postmenopausal women	13.1 $\pm$ 6.8 U/ml	[39]
Expected tumor cell density in solid tumor tissue	2 $\times$ 10^5/mm^3	[20]

Note: Mean cell density in solid (tumor) tissue = 10^6/mm^3 [20]. To account for the fact that tumor tissue contains other cells besides tumor cells and that ovarian tumors tend to have cystic components: only 20% of mean total number of cells in tissue volume were considered to be actual tumor cells = 2 $\times$ 10^5/mm^3.

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be used to relate tumor size to blood biomarker levels. To further understand limitations of this model, a sensitivity analysis was performed assuming variable physiologic conditions of the model parameters. For this purpose, the model parameters were primed on the basis of data in the literature for two established and well-studied serum tumor biomarkers, CA125 for ovarian cancer and PSA for prostate cancer. Through this approach, the potential minimal tumor size could be calculated that leads to CA125/PSA tumor biomarker levels in serum detectable by standard clinical or (in the case of CA125) future hypothetical more sensitive experimental proteomics blood tests.

General Parameters and Variables of the Tumor Biomarker Model

The general parameters and variables of the serum tumor biomarker model are listed in Tables 1 and 2 (CA125) as well as Tables 3 and 4 (PSA).

CA125

The reported sensitivities for clinically available CA125 II immunoassays range between detection limits of 0.05 and 1.45 U/ml [6,21]. On the other end of the spectrum are CA125 ELISA assays that are used for research with detection limits of 5 U/ml [22]. To account for the possibility that newer, more sensitive proteomics test assays will be developed, a detection limit as low as 0.01 U/ml was also considered. The cut-off level for distinction between healthy and disease depends on whether the biomarker is secreted by normal cells (background secretion) or by tumor cells only. If the tumor biomarker is secreted by tumor cells only, the test assay sensitivity basically defines the cut-off between healthy and disease. If there is secretion by healthy cells, the cut-off value for distinction between healthy and disease has to be set at a point that offers a high specificity and beyond which as few of the normal population as possible will lie. Therefore, in order to include less than 0.1% of the normal population to the range of positive test results assuming a Gaussian distribution of the CA125 levels, the cut-off level was set at 3.09 times the standard deviations above the mean normal protein serum level in healthy women (34.11 U/ml). Setting it, e.g., at three times the standard deviations above the mean value would be slightly to low. The cut-off level (or more precisely how many times the standard deviations above the mean normal protein serum level the cut-off level is set) defines the percent of test

Table 2. Variables Used to Test the Tumor Biomarker Assay Model CA125

Variable	Baseline	Range	References
Sensitivity of blood proteomics test	1.45 U/ml	0.01–5 U/ml	[6,21,22]
Cut-off level for distinction between healthy and disease	—	0.01–34.11 U/ml	[6,8,21,22]
Percentage of false positive test assay results	0.1 %	0.1%–5%	—
Fraction of secreted protein reaching plasma	10 %	0.1%–20%	—
Tumor biomarker secretion rate by tumor cells	—	2–130 U/10^5 cells/20 h/ml	[6]

Note: In order to account for potential field effects of the tumor microenvironment on the biomarker secretion rates we chose a broader range of CA125 secretion rates up to 10-fold higher than those published in the literature.
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results of the healthy population that will be counted to the positive test results. However, to account for different test situations, a sensitivity analysis with regard to varying probabilities of false positive test results was performed. Therefore, a total range of 0.01–34.11 U/ml was used as cut-off level.

Not all of the protein secreted by the cells into the extracellular fluid compartment will reach the intravascular space. A range of 0.1%–20% mean percentages of secreted tumor biomarker getting into the intravascular space was assumed.

The secretion rate of CA125 into cell culture medium by selected ovarian cancer cell lines (OVCAR-3, SK-OV-8, SK-OV-3 secreting 2–13 U/10^5 cells/20 h/ml medium) has been reported by Zeimet et al [6]. As the results of the study by Zeimet et al. represent cell culture data, a wider range of secretion rates (up to 130 U/10^5 cells/20 h/ml medium) was used for the model to account for potential enhancing influences of the tumor microenvironment (field effects) on the actual tumor biomarker secretion.

PSA

The reported sensitivities for clinically available PSA immunoassays range between detection limits of 0.01 ng/ml and 0.1 ng/ml [14,23,24]. As mentioned before, the cut-off level for the distinction between healthy and disease depends on whether the blood biomarker is secreted by normal cells or by tumor cells only. If there were no background secretion of PSA by normal cells, the highly sensitive immunoassays that are clinically available for PSA would allow for very low cut-off levels. However, there is background secretion of PSA by nonmalignant prostate cells. PSA serum values are known

to increase with increasing prostate volumes as in benign prostate hyperplasia, a condition that is highly prevalent in older men [25]. In screening for prostate cancer, the American Urological Association recommends to proceed to prostate biopsy in patients exceeding the PSA cut-off level 4 ng/ml [26]. Some authors even recommend prostate biopsies at PSA levels of 2.6–4 ng/ml in men younger than 60 y [27]. Up to 25% of the more aggressive prostate cancer types are found in men with PSA levels below 4 ng/ml, but despite this fact there is fear that lowering the cut-off level might result in overdiagnosing and overtreatment of prostate cancer [17]. For our model, therefore, we chose the widely recommended cut-off level of 4 ng/ml to differentiate between healthy and disease state. This value is actually close to a cut-off level calculated by adding 3.09 × the standard deviation to the mean PSA level in healthy 50- to 59-y-old men (4.161 ng/ml) [28] . Since normal values of PSA are age dependent, we used a published normal value of 1.38 ng/ml for the age group of 50- to 59-y-old men [28].

A range of 0.1%–20% of secreted tumor biomarker getting into the intravascular space was assumed.

Reported PSA secretion rates by the prostate cancer cell line LNCaP are 21 ng/10^5 cells/ml/24 h and 134 ng/10^6 cells/ml/24 h, respectively [29,30]. In order to account for tumor cell types that might secrete lower amounts of PSA and for potential influences on the cell secretion by the tumor microenvironment (field effects), we chose a broader range of secretion rates for our calculations and added secretion rates as low as 2.1 ng/10^5 cells/ml/24 h and 10-fold higher than the published value of 134 ng/10^6 cells/ml/24 h (up to a maximum of 1,340 ng/10^6 cells/ml/24 h) to the analysis.

Results

Using a linear one-compartment model, the tumor biomarker level in patient plasma under steady state conditions was described as a function of either tumor biomarker secretion by tumor cells or, if applicable, by healthy normal cells (influx) and tumor biomarker plasma half-life (efflux) (Figure 1).

CA125 Secretion by Tumor Cells Only

When no biomarker secretion by healthy cells was assumed, the required tumor size resulting in detectable CA125 levels was defined by the actual detection limit or sensitivity of the proteomics assay (model variables, Table 2). In that case, under the assumption of a percentage of 10% of the secreted tumor biomarker reaching the intravascular space, the

Table 3. General Parameters of the Tumor Biomarker Model PSA

Parameter	Value	Reference
Mean plasma/serum volume in a 85-kg-male patient	3,825 ml	[31]
Serum half-life of serum tumor biomarker PSA	46.08 h	[45]
Mean serum level of tumor biomarker (PSA) in healthy men, age 50–59 y	1.38 ± 0.9 ng/ml	[28]
Expected tumor cell density in solid tumor tissue	5 × 10^5/mm^3	[20]

Note: Mean cell density in solid (tumor) tissue = 10^6/mm^3 [20]. To account for the fact that tumor tissue contains other cells besides tumor cells: only 50% of mean total number of cells in tissue volume were considered to be actual tumor cells = 5 × 10^5/mm^3.
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Table 4. Variables Used to Test the Tumor Biomarker Assay Model PSA

Variable	Baseline	Range	References
Sensitivity of blood proteomics test	0.04 ng/ml	0.01–0.1 ng/ml	[14,23,24]
Cut-off level for distinction between healthy and disease	4 ng/ml	—	[26]
Fraction of secreted PSA reaching plasma	10%	0.1%–20%	—
Tumor biomarker secretion rate by tumor cells	—	2.1–134 ng/10^5 cells/24 h/ml	[29,30]

Note: In order to account for potential field effects of the tumor microenvironment on the biomarker secretion rates we chose a broader range of PSA secretion rates (up to 10-fold lower and higher than published in the literature).
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calculated tumor sizes leading to detectable tumor biomarker levels in patients' plasma/serum ranged between 0.11 mm^3 and 3,610.14 mm^3 depending on the assumed tumor cell secretion rates and sensitivity of the blood assay (Table 5). Under the assumption of an assay sensitivity of 1.45 U/ml (clinically realistic conditions) and 10% of tumor biomarker reaching the plasma/serum, the detectable tumor sizes ranged between 16.11 mm^3 and 1,046.94 mm^3.

CA125 Secretion by Tumor Cells and Healthy Cells

When biomarker secretion by healthy cells is present (a more likely scenario), it was assumed that the secretion of CA125 by healthy cells accounts for up to 13.1 U/ml, and that tumor cells contribute the rest of the secretion up to the required cut-off level. The calculated tumor biomarker secretion by healthy cells was 0.06 U/ml/h, and the secretion rate by tumor cells was 0.10 U/ml/h. Extrapolated to the total body plasma volume (3,150 ml) the tumor biomarker secretion rate by healthy cells and by tumor cells was 189.17 U/3,150 ml/h and 303.43 U/3,150 ml/h, respectively, assuming a test assay cut-off level of 34.11 U/ml. Taking into account the varying tumor biomarker secretion rates, the calculated tumor sizes leading to positive test results (over the cut-off level of 34.11 U/ml patient serum) ranged between 116.7 mm^3 and 1.52×10^6 mm^3 (1.52 l) under the assumption of 10% of the secreted tumor biomarker reaching the plasma (Table 6).

CA125—Influence of Protein Assay Cut-off Level/Test Specificity on Tumor Size

Assuming a Gaussian distribution of protein tumor biomarker levels in healthy controls, 10% of the secreted

tumor biomarker reaching the plasma, and that the minimum detection limit of the assay is 1.45 U/ml, the minimally detectable tumor sizes ranged between 123.88 and 15,171.26 mm^3 depending on the chosen probability of false positive test results (Table 7).

PSA Secretion by Tumor Cells Only

When no biomarker secretion by healthy cells was assumed, the required tumor size resulting in detectable PSA levels was defined by the actual detection limit or sensitivity of the proteomics assay (model variables, Table 4). Under the assumption of 10% of the secreted tumor biomarker reaching the intravascular space, the calculated tumor sizes leading to detectable PSA levels in patients' serum ranged between 0.21 mm^3 and 131.51 mm^3 depending on the assumed tumor cell secretion rates and sensitivity of the blood assay (Table 8). When an assay sensitivity of 0.04 ng/ml (clinically realistic conditions) and 10% of tumor biomarker reaching the plasma/serum were assumed, the detectable tumor sizes ranged between 0.82 mm^3 and 52.6 mm^3. Even an assay sensitivity of 0.01 ng/ml would still be clinically realistic and then decrease the detection levels to a range of 0.21 to 13.15 mm^3 under the assumption of 10% of the secreted PSA reaching the intravascular space.

PSA Secretion by Tumor Cells and Healthy Cells

PSA is also secreted to some extent by nonmalignant cells. When biomarker secretion by healthy cells is present, it was assumed that the secretion of PSA by healthy cells accounts for 1.38 ng/ml (normal value for 50- to 59-y-old men) and secretion by tumor cells accounts for the remaining 2.62 ng/ml up to the cut-off level of 4 ng/ml. The calculated

Table 5. Minimum Detectable Tumor Cell Number and Estimated Tumor Size (in mm^3) as a Function of CA125 Proteomics Test Assay Sensitivity

Biomarker Secretion Rate	Detection Limit of Proteomics Test Assay				
	0.01 U/ml	0.05 U/ml	0.1 U/ml	1.45 U/ml	5 U/ml
Increased tumor cell secretion in tumor microenvironment	2.22×10^4	1.11×10^5	2.22×10^5	3.22×10^6	1.11×10^7
(6.5 U/ml/h/10^5 cells); 10% of tumor marker getting into plasma	(0.11 mm^3)	(0.56 mm^3)	(1.11 mm^3)	(16.11 mm^3)	(55.54 mm^3)
Tumor cells secreting high amounts of tumor marker	2.22×10^5	1.11×10^6	2.22×10^6	3.22×10^7	1.11×10^8
(0.65 U/ml/h/10^5 cells); 10% of tumor marker getting into plasma	(1.11 mm^3)	(5.55 mm^3)	(11.11 mm^3)	(161.07 mm^3)	(555.41 mm^3)
Tumor cells secreting intermediate amounts of tumor marker	3.21×10^5	1.6×10^6	3.21×10^6	4.65×10^7	1.6×10^8
(0.45 U/ml/h/10^5 cells); 10% of tumor marker getting into plasma	(1.60 mm^3)	(8.02 mm^3)	(16.05 mm^3)	(232.65 mm^3)	(802.25 mm^3)
Tumor cells secreting low amounts of tumor marker	1.44×10^6	7.22×10^6	1.44×10^7	2.09×10^8	7.22×10^8
(0.1 U/ml/h/10^5 cells); 10% of tumor marker getting into plasma	(7.2 mm3)	(36.10 mm^3)	(72.20 mm^3)	(1,046.94 mm^3)	(3,610.14 mm^3)

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Table 6. Minimum Detectable Tumor Cell Number and Estimated Tumor Size (Size in mm³) as a Function of CA125 Background Secretion and Percentage of CA125 Reaching the Plasma

Biomarker Secretion Rate	No Background Secretion by Healthy Cells					Background Secretion by Healthy Cells Present				
	Percentage of Tumor Biomarker Reaching Plasma					Percentage of Tumor Biomarker Reaching Plasma				
	0.1%	1%	5%	10%	20%	0.1%	1%	5%	10%	20%
Increased secretion rate in tumor microenvironment (6.5 U/ml/h/10^5 cells); minimum detection limit of proteomics test 1.45 U/m	3.22×10^8 (1,610.68 mm³)	3.22×10^7 (161.07 mm³)	6.44×10^6 (32.21 mm³)	3.22×10^6 (16.11 mm³)	1.61×10^6 (8.05 mm³)	4.67×10^9 (23,340.4 mm³)	4.67×10^8 (2,334.04 mm³)	9.34×10^7 (466.81 mm³)	4.67×10^7 (233.4 mm³)	2.33×10^7 (116.70 mm³)
Tumor cells secreting high amounts of tumor marker (0.65 U/ml/h/10^5 cells); minimum detection limit of proteomics test 1.45 U/m	3.22×10^9 (16,106.79 mm³)	3.22×10^8 (1,610.68 mm³)	6.44×10^7 (322.14 mm³)	3.22×10^7 (161.07 mm³)	1.61×10^7 (80.53 mm³)	4.67×10^{10} (2.33×10^5 mm³)	4.67×10^9 (23,340.4 mm³)	9.34×10^8 (4,668.08 mm³)	4.67×10^8 (2,334.04 mm³)	2.33×10^8 (1,167.02 mm³)
Tumor cells secreting intermediate amounts of tumor marker (0.45 U/ml/h/10^5 cells); minimum detection limit of proteomics test 1.45 U/m	4.65×10^9 (23,265.36 mm³)	4.65×10^8 (2,326.54 mm³)	9.31×10^7 (465.31 mm³)	4.65×10^7 (232.65 mm³)	2.33×10^7 (116.33 mm³)	6.74×10^{10} (3.37×10^5 mm³)	6.74×10^9 (33,713.91 mm³)	1.35×10^9 (6,742.78 mm³)	6.74×10^8 (3,371.39 mm³)	3.37×10^8 (1,685.7 mm³)
Tumor cells secreting low amounts of tumor marker (0.1 U/ml/h/10^5 cells); minimum detection limit of proteomics test 1.45 U/m	2.09×10^{10} (1.05×10^5 mm³)	2.09×10^9 (10,469.41 mm³)	4.19×10^8 (2,093.88 mm³)	2.09×10^8 (1,046.94 mm³)	1.05×10^8 (523.47 mm³)	3.03×10^{11} (1.52×10^6 mm³)	3.03×10^{10} (1.52×10^5 mm³)	6.07×10^9 (30,342.52 mm³)	3.03×10^9 (15,171.26 mm³)	1.52×10^9 (7,585.63 mm³)

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biomarker secretion rate by tumor cells was 0.04 ng/ml/h. Extrapolated to the total body plasma volume (3,825 ml) the biomarker secretion rate by tumor cells was 150.75 ng/3,825 ml/h assuming a test assay cut-off level of 4 ng/ml. Taking into account the varying tumor biomarker secretion rates, the calculated tumor sizes leading to positive test results (over the cut-off level of 4 ng/ml patient serum) ranged between 27 mm³ and 3.45×10^5 mm³ (0.35 l) under the assumption of 10% of the secreted tumor biomarker reaching the plasma (Table 9).

Discussion

The aim of this study was to relate serum tumor biomarker levels as detected by a blood assay to detectable tumor sizes, because such a relationship can likely aid in determining the lower limit for detectable tumor sizes. A linear one-compartment model (Figure 1) was used to describe the pharmacokinetics of a protein serum tumor biomarker in blood, including tumor biomarker secretion by tumor cells (and healthy cells, if background biomarker secretion is also present), transfer to the intravascular space, and final degradation and/or removal from the plasma (represented as outflow [F_{out}] in the model). By priming the model parameters on the basis of the literature data for two established and well-studied tumor biomarkers, CA125 for ovarian cancer and PSA for prostate cancer, the potential detection limits needed for a proteomic blood test with regards to tumor burden were calculated under varying physiological and assay conditions (Tables 1–4) [6–8,14,20–24,26,28–31]. A sensitivity analysis of the model (by varying model parameters over a range of parameter assumptions) resulted in a wide range of minimally detectable tumor sizes depending on the specific chosen conditions. In the ideal case scenario of a novel serum tumor biomarker that is secreted only by tumor cells, depending on the chosen parameters (tumor biomarker secretion rate, its fraction reaching the plasma, and the available blood test assay sensitivity), very small ovarian cancer lesions down to a size of 0.11–7.22 mm³ may be detected with a highly sensitive blood assay. Assuming the same ideal case scenario of exclusive biomarker secretion by tumor cells for prostate cancer, comparably small lesions in a range of 0.21–13.15 mm³ may be detectable even with a clinically available PSA immunoassay (detection limit 0.01 ng/ml). In a clinically more realistic scenario, however, with additional secretion of the serum tumor biomarker by healthy cells, ovarian cancer lesions between 116.7 mm³ and 1.52×10^6 mm³ (1.52 l), roughly corresponding to a size range between 4.89^3 mm³ and 114.98^3 mm³, may be detected depending on the chosen physiological and model parameters.

In our study, the minimal tumor lesion sizes obtained by the sensitivity analysis under the assumption of tumor biomarker secretion by healthy cells (it is known that healthy mesothelial cells secrete CA125 [6]) ranged between several hundred mm³ and more than 10^6 mm³ for ovarian cancer. Indeed, these results are in qualitative agreement with the performance of CA125 in clinical applications. CA125 tests are positive in up to 80% of patients with advanced stage disease (e.g., tumor volumes up to 10^6 mm³ are reported for advanced ovarian cancer with peritoneal metastases [32]), but are negative in up to 50% of patients with stage I disease

Table 7. Minimum Detectable Tumor Cell Number and Estimated Tumor Size (in mm^3) as a Function of CA125 Proteomics Test Assay Cut-Off Level/Specificity of Test Results

Biomarker Secretion Rate	Percentage of False Positive Test Results (Cut-Off Level)			
	5% (24.25)	2% (27.04)	1% (28.94)	0.1% (34.11)
Increased tumor cell secretion in tumor microenvironment (6.5 U/ml/h/10^5 cells); 10% of tumor marker getting into plasma; minimum detection limit of proteomics test 1.45 U/ml	2.48 × 10^7 (123.88 mm^3)	3.1 × 10^7 (154.85 mm^3)	3.52 × 10^7 (176.0 mm^3)	4.67 × 10^7 (233.4 mm^3)
Tumor cells secreting high amounts of tumor marker (0.65 U/ml/h/10^5 cells); 10% of tumor marker getting into plasma; minimum detection limit of proteomics test 1.45 U/ml	2.48 × 10^8 (1,238.78 mm^3)	3.1 × 10^8 (1,548.47 mm^3)	3.52 × 10^8 (1,759.97 mm3)	4.68 × 10^8 (2,334.04 mm^3)
Tumor cells secreting intermediate amounts of tumor marker (0.45 U/ml/h/10^5 cells); 10% of tumor marker getting into plasma; minimum detection limit of proteomics test 1.45 U/ml	3.58 × 10^8 (1,789.35 mm^3)	4.47 × 10^8 (2,236.68 mm^3)	5.08 × 10^8 (2,542.18 mm^3)	6.74 × 10^8 (3,371.39 mm^3)
Tumor cells secreting low amounts of tumor marker (0.1 U/ml/h/10^5 cells); 10 % of tumor marker getting into plasma; minimum detection limit of proteomics test 1.45 U/ml	1.61 × 10^9 (8,052.06 mm^3)	2.01 × 10^9 (10,065.07 mm^3)	2.29 × 10^9 (11,439.82 mm^3)	3.03 × 10^9 (15,171.26 mm^3)

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when the tumor burden is still limited [33–35]. Likewise, in a clinically more realistic scenario for PSA with background secretion of PSA also by healthy prostatic cells, we found that tumor lesions ranged in size between 27 mm^3 and 3.45 × 10^5 mm^3 (0.35 l), which approximately corresponds to a size range between 3^3 mm^3 and 70.14^3 mm^3 that can be detected by a serum marker test. Again, these size ranges largely depend on the physiological and model parameters chosen for the model. In the clinic, PSA serum levels are known to positively correlate with prostate cancer size. However, even at low PSA levels of 4 ng/ml, a tumor as big as 10,000 mm^3 can already be present, underlining the large physiological variability of tumor marker secretion by tumors in patients [36].

When biomarker secretion by tumor and normal cells were assumed and sensitivities of clinically available proteomics assays were used for our model analysis, the calculated minimally detectable tumor sizes were smaller for PSA than for CA125 in our study, but still in a comparable range. This may seem surprising, given the much lower cut-off level for differentiation between healthy and disease state for PSA (4 ng/ml) compared with CA125 (34.11 U/ml), the chosen higher tumor cell density for prostate tumors than for ovarian tumors and the comparable range of both biomarker secretion rates. The different measurement units (units/ml

for CA125 versus ng/ml for PSA) do not actually matter in this mathematical model, since they cancel each other out during the calculations. However, the plasma half-life is much shorter for PSA than for CA125 (46.08 h for PSA versus 151.2 h for CA125), and the chosen average plasma volume is much larger for men than for women (3,825 ml for men versus 3,150 ml for women) [31]. Therefore, because of the shorter PSA plasma half-life as well as the larger plasma volume in men, more PSA has to be secreted by prostate tumors to reach a steady state level detectable with proteomics assays. These facts may explain why the calculated minimally detectable tumor sizes were within a comparable range for both biomarkers despite the fact that PSA has a lower cut-off level than CA125.

We acknowledge that CA125 and PSA are not ideal cancer screening biomarkers. CA125 is considered a valuable biomarker for assessing therapeutic success in ovarian cancer patients rather than being used as a diagnostic screening biomarker [37]. PSA is often considered too sensitive and not specific enough for screening purposes of prostate cancer since the number of false positive results is too high for PSA. Increased PSA levels are also found at high percentages in patients with benign prostatic hypertrophy, a disorder with high prevalence in the older male population [38]. Accord-

Table 8. Minimum Detectable Tumor Cell Number and Estimated Tumor Size (in mm^3) as a Function of PSA Proteomics Test Assay Sensitivity

Biomarker Secretion Rate	Detection Limit of Proteomics Test Assay			
	0.01 ng/ml	0.04 ng/ml	0.08 U/ml	0.1 ng/ml
Increased tumor cell secretion in tumor microenvironment (5.58 ng/ml/h/10^5 cells); 10% of tumor marker getting into plasma	1.03 × 10^5 (0.21 mm^3)	4.12 × 10^5 (0.82mm^3)	8.24 × 10^5 (1.65 mm^3)	1.03 × 10^6 (2.06 mm^3)
Tumor cells secreting high amounts of tumor marker (0.88 ng/ml/h/10^5 cells); 10% of tumor marker getting into plasma	6.58 × 10^5 (1.32 mm^3)	2.63 × 10^6 (5.26mm^3)	5.26 × 10^6 (10.52 mm^3)	6.58 × 10^6 (13.15 mm^3)
Tumor cells secreting intermediate amounts of tumor marker (0.56 ng/ml/h/10^5 cells); 10% of tumor marker getting into plasma	1.03 × 10^6 (2.06 mm^3)	4.12 × 10^6 (8.24 mm^3)	8.24 × 10^6 (16.49 mm^3)	1.03 × 10^7 (20.61 mm^3)
Tumor cells secreting low amounts of tumor marker (0.09 ng/ml/h/10^5 cells); 10% of tumor marker getting into plasma	6.58 × 10^6 (13.15 mm^3)	2.63 × 10^7 (52.6 mm^3)	5.26 × 10^7 (105.21 mm^3)	6.58 × 10^7 (131.51 mm^3)

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Table 9. Minimum Detectable Tumor Cell Number and Estimated Tumor Size (Size in mm³) as a Function of PSA Background Secretion and Percentage of PSA Reaching the Plasma

Biomarker Secretion Rate	No Background Secretion by Healthy Cells					Background Secretion by Healthy Cells Present				
	0.1%	1%	5%	10%	20%	0.1%	1%	5%	10%	20%
Increased tumor cell secretion in tumor microenvironment (5.58 ng/ml/h/10^5 cells); minimum detection limit of proteomics test 0.04 ng/ml	4.12×10^7 (82.44 mm³)	4.12×10^6 (8.24 mm³)	8.24×10^5 (1.65 mm³)	4.12×10^5 (0.82 mm³)	2.06×10^5 (0.41 mm³)	2.7×10^9 (5,399.86 mm³)	2.7×10^8 (539.99 mm³)	5.4×10^7 (108.00 mm³)	2.7×10^7 (54.00 mm³)	1.35×10^7 (27.00 mm³)
Tumor cells secreting high amounts of tumor marker (0.88 ng/ml/h/10^5 cells); minimum detection limit of proteomics test 0.04 ng/ml	2.63×10^8 (526.05 mm³)	2.63×10^7 (52.60 mm³)	5.26×10^6 (10.52 mm³)	2.63×10^6 (5.26 mm³)	1.32×10^6 (2.63 mm³)	1.72×10^{10} (34,456.22 mm³)	1.72×10^9 (3,445.62 mm³)	3.45×10^8 (689.12 mm³)	1.72×10^8 (344.56 mm³)	8.61×10^7 (172.28 mm³)
Tumor cells secreting intermediate amounts of tumor marker (0.56 ng/ml/h/10^5 cells); minimum detection limit of proteomics test 0.04 ng/ml	4.12×10^8 (824.41 mm³)	4.12×10^7 (82.44 mm³)	8.24×10^6 (16.49 mm³)	4.12×10^6 (8.24 mm³)	2.06×10^6 (4.12 mm³)	2.7×10^{10} (53,998.56 mm³)	2.7×10^9 (5,399.86 mm³)	5.4×10^8 (1,079.97 mm³)	2.7×10^8 (539.99 mm³)	1.35×10^8 (269.99 mm³)
Tumor cells secreting low amounts of tumor marker (0.09 ng/ml/h/10^5 cells); minimum detection limit of proteomics test 0.04 ng/ml	2.63×10^9 (5,260.49 mm³)	2.63×10^8 (526.05 mm³)	5.26×10^7 (105.21 mm³)	2.63×10^7 (52.60 mm³)	1.32×10^7 (26.30 mm³)	1.72×10^{11} (3.45×10^5 mm³)	1.72×10^{10} (34,456.22 mm³)	3.45×10^9 (6,891.24 mm³)	1.72×10^9 (3,445.62 mm³)	8.61×10^8 (1,722.81 mm³)

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ingly, "normal" PSA levels are closely linked to age and prostate gland size, which is reflected by increasing PSA serum levels with increasing age [28]. On the other hand, high grade cancers, which have an aggressive clinical course with early metastasis and, thus, need to be detected at very early stage for a curative treatment, can present with PSA levels smaller than 4 ng/ml in up to 25% of cases [17]. However, we chose these two serum tumor biomarkers for our study because both markers are among the most widely studied tumor biomarkers and many physiological parameters such as in vitro secretion rates in tumor cell culture studies, blood half-life in healthy men and women, and normal serum levels in cohorts of healthy individuals have been published in the literature and can be applied in the our model. The choice of these biomarkers is not because we in any way advocate their use in early cancer detection, but as stated above, because not enough data exist on almost all biomarkers relevant for the type of modeling performed in our study.

For PSA we performed the sensitivity analysis assuming the clinically established and widely recommended cut-off level of 4 ng/ml for differentiating between presence of tumor and no tumor [26]. In general, for a novel biomarker the cut-off level of a novel biomarker test assay has to be optimized to reduce the number of false positive results causing unnecessary follow-up examinations or therapies following a positive screening test. Therefore, a wide safety margin above the mean level in healthy controls is usually chosen to increase the assay specificity. For CA125 in our study, we set the cut-off level at 3.09 times the standard deviation (at 34.11 U/ml) above the mean CA125 value found in healthy postmenopausal women (13.1 U/ml), thereby excluding 99.9% of healthy individuals from false positive test results in our model. The cut-off value of 34.11 U/ml in our study is actually very close to the cut-off limit of 35 U/ml that was originally reported for CA125 tests to differentiate benign from malignant disease in patients [39]. With our mathematical model we are also able to show the influence of test specificity on potential detection limits of a tumor blood biomarker assay. However, this demonstration does not take into account that very high test specificity may result in low test sensitivity and thus result in a high likelihood of false-negative test results. In order to calculate sensitivity, accuracy, and positive/negative predictive values of a blood biomarker assay, however, the mean level and standard deviation of the diseased population's blood tumor biomarker would need to be further specified.

Extensive research in the field of biomarkers for early detection of ovarian cancer is currently underway with many promising candidates emerging over the last few years [1]. Our model clearly points out that blood biomarkers that are specific for a malignant disease are more desirable than nonspecific biomarkers that are also secreted by nonmalignant cells. For example, in the ideal scenario of a highly specific biomarker that is not secreted by healthy cells there is the potential to detect early-stage tumors as small as 0.11–13.15 mm³ on the basis of our calculations. In addition, a combination of two or more highly specific tumor biomarkers may further increase performance of biomarker screening for ovarian cancer, especially if they complement each other. It is expected that the currently available biomarker CA125 may be used as part of a panel of serum tumor biomarkers [40,41]. Actually our model may even be

extended to accommodate the concept of a biomarker panel by combining multiple one-compartment models each with distinct parameters for a given distinct biomarker protein. Furthermore, our model is not limited to biomarkers for ovarian or prostate cancer and can be applied to any type of tumor secreting distinctive biomarkers that find access to the bloodstream and that can be measured in patient serum samples.

The following limitations of our model need to be addressed. To the best of our knowledge, there are no quantitative values available in the literature on the amount of secreted tumor biomarker per cancer cell in intact whole body animal tumor models or in patients with any type of cancer. In addition, the fraction of secreted tumor biomarkers actually reaching the intravascular space in animal tumor models or in patients with cancer has not been determined in the literature to date. Therefore, we used data from published cell culture experiments to estimate tumor cell secretion rates of the biomarker, and we assumed a wide range of secretion rates to account for potential changes in tumor biomarker secretion rates due to the effects of the tumor environment in intact living individuals. Furthermore, the tumor biomarker secretion rate from tumor and/or normal healthy cells may change over time in a patient with cancer causing the tumor biomarker plasma concentration to vary at different time points (e.g., circadian variation). A modification of the linear one-compartment flow model (comparable to the principle of repeated extravascular dosing [42]) may account for time-dependent changes of tumor biomarker secretion and is currently under investigation. As soon as more detailed data on these varying physiologic processes in patients with ovarian, prostate, or other cancer become available in the literature, our model could be modified to account for such time-dependent changes in protein secretion rates. Furthermore, the model is suitable for the wide variety of secreted protein blood markers, but not for other important novel classes of blood tumor biomarkers such as auto-antibodies that are targeted against tumor antigens and can be found in patient blood samples. Currently, the model also does not account for changes in the tumor microenvironment that might occur very early in a developing cancer when there might be nonsecreted biomarkers present (e.g., biomarkers that get cleaved from tumor cells by interaction with proteases) [43,44].

In conclusion, in this study we introduced a linear one-compartment mathematical model that allows estimation of minimal detectable tumor sizes on the basis of blood tumor biomarker assays. Assuming physiological data on CA125 and PSA from the literature, the reported mathematical model predicted detection limits of ovarian and prostate tumors that are in qualitative agreement with the actual clinical performance of the biomarkers. Our mathematical model may be helpful in future estimation of minimal detectable tumor sizes for novel proteomic biomarker assays if sufficient physiologic data for the biomarker are available. Important basic studies that scientists in the cancer diagnostics field should consider when exploring the potential of novel biomarkers include biomarker testing of secretion rates in cell culture studies and testing of biomarker half-life in patients undergoing treatment. Future experiments in (animal) models of biomarker secretion such as reporter models may reveal the required data for in vivo biomarker cell secretion rates, influence of tumor microenvironment on biomarker secretion rates, and the percentage of tumor marker reaching the intravascular space. A reporter model, e.g., with xenografts/allografts of tumor cell lines that are modified to secrete a unique experimental "biomarker" may be helpful to relate tumor sizes to biomarker blood levels more reliably. Furthermore, models in which secretion of a biomarker is coupled to simultaneous expression/secretion of a fluorescent dye may help characterize the extents of secreted biomarkers reaching the intravascular space. It is not easy to develop and implement these models, but they are potentially very important since they may help us understand the physiological processes of in vivo biomarker cell secretion.

Acknowledgments

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Editors' Summary

Background. Cancers—disorganized masses of cells that can occur in any tissue—develop when cells acquire genetic changes that allow them to grow uncontrollably and to spread around the body (metastasize). If a cancer (tumor) is detected when it is small, surgery can often provide a cure. Unfortunately, many cancers (particularly those deep inside the body) are not detected until they are large enough to cause pain or other symptoms by pressing against surrounding tissue. By this time, it may be impossible to remove the original tumor surgically and there may be metastases scattered around the body. In such cases, radiotherapy and chemotherapy can sometimes help, but the outlook for patients whose cancers are detected late is often poor. Consequently, researchers are trying to develop early detection tests for different types of cancer. Many tumors release specific proteins—"cancer biomarkers"—into the blood and the hope is that it might be possible to find sets of blood biomarkers that detect cancers when they are still small and thus save many lives.

Why Was This Study Done? For most biomarkers, it is not known how the amount of protein detected in the blood relates to tumor size or how sensitive the assays for biomarkers must be to improve patient survival. In this study, the researchers develop a "linear one-compartment" mathematical model to predict how large tumors need to be before blood biomarkers can be used to detect them and test this model using published data on two established cancer biomarkers—CA125 and prostate-specific antigen (PSA). CA125 is used to monitor the progress of patients with ovarian cancer after treatment; ovarian cancer is rarely diagnosed in its early stages and only one-fourth of women with advanced disease survive for 5 y after diagnosis. PSA is used to screen for prostate cancer and has increased the detection of this cancer in its early stages when it is curable.

What Did the Researchers Do and Find? To develop a model that relates secreted blood biomarker levels to tumor sizes, the researchers assumed that biomarkers mix evenly throughout the patient's blood, that cancer cells secrete biomarkers into the fluid that surrounds them, that 0.1%–20% of these secreted proteins enter the blood at a continuous rate, and that biomarkers are continuously removed from the blood. The researchers then used their model to calculate the smallest tumor sizes that might be detectable with these biomarkers by feeding in existing data on CA125 and on PSA, including assay detection limits and the biomarker secretion rates of cancer cells growing in dishes. When only tumor cells secreted the biomarker and 10% of the secreted biomarker reach the blood, the model predicted that ovarian tumors between 0.11 mm^3 (smaller than a grain of salt) and nearly 4,000 mm^3

(about the size of a cherry) would be detectable by measuring CA125 blood levels (the range was determined by varying the amount of biomarker secreted by the tumor cells and the assay sensitivity); for prostate cancer, the detectable tumor sizes ranged from similar lower size to about 130 mm^3 (pea-sized). However, healthy cells often also secrete small quantities of cancer biomarkers. With this condition incorporated into the model, the estimated detectable tumor sizes (or total tumor burden including metastases) ranged between grape-sized and melon-sized for ovarian cancers and between pea-sized to about grapefruit-sized for prostate cancers.

What Do These Findings Mean? The accuracy of the calculated tumor sizes provided by the researchers' mathematical model is limited by the lack of data on how tumors behave in the human body and by the many assumptions incorporated into the model. Nevertheless, the model predicts detection limits for ovarian and prostate cancer that broadly mirror the clinical performance of both biomarkers. Somewhat worryingly, the model also indicates that a tumor may have to be very large for blood biomarkers to reveal its presence, a result that could limit the clinical usefulness of biomarkers, especially if they are secreted not only by tumor cells but also by healthy cells. Given this finding, as more information about how biomarkers behave in the human body becomes available, this model (and more complex versions of it) should help researchers decide which biomarkers are likely to improve early cancer detection and patient outcomes.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.0050170.

- The US National Cancer Institute provides a brief description of what cancer is and how it develops and a fact sheet on tumor markers; it also provides information on all aspects of ovarian and prostate cancer for patients and professionals, including information on screening and testing (in English and Spanish)
- The UK charity Cancerbackup also provides general information about cancer and more specific information about ovarian and prostate cancer, including the use of CA125 and PSA for screening and follow-up
- The American Society of Clinical Oncology offers a wide range of information on various cancer types, including online published articles on the current status of cancer diagnosis and management from the educational book developed by the annual meeting faculty and presenters. Registration is mandatory, but information is free