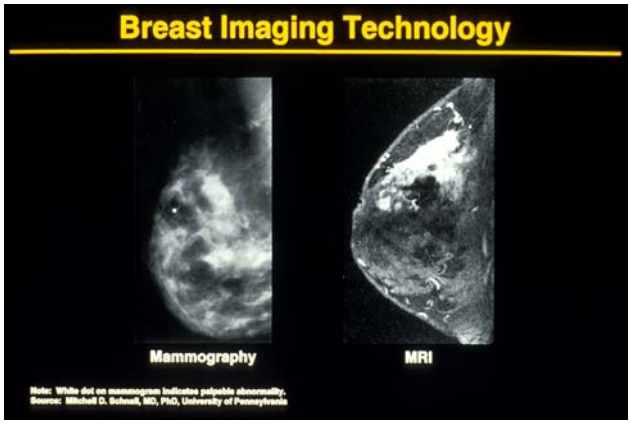
Chapter 10

Technologies for Early Detection and Prevention of Cancer

In Chapter 8, we saw how technology could be used to prevent infectious diseases, one of the leading killers in the developing world. In this unit, we examine how technology can be used to diagnose disease. Our focus is the detection of cancer, where early detection (**Figure 10.1**) can mean the difference between life and death. We begin by examining the global burden of cancer. Next, we examine how cancers develop and why early detection is so crucial. Finally, we examine three cancers in detail – cervical cancer, ovarian cancer and prostate cancer – and look at existing and new technologies to aid in the early detection and prevention of each disease.



Source:Mitchell D. Schnall, M.D., Ph.D. University Of Pennsylvania, National Cancer Institute

Figure 10.1: Mammography is one method used to screen for breast cancer.

The burden of cancer in the United States

Cancer is the second leading cause of death in the United States, responsible for nearly 1 out of every 4 deaths (**Table 10.1**). Only 66% of all cancer patients live more than 5 years past their initial diagnosis, a statistic known as the 5-year survival rate. Cancer is important from an economic perspective as well. In the US alone, cancer cost approximately $206 billion in 2006. $78.2 billion of this was spent on direct medical costs; $17.9 billion represents lost productivity to illness and $110.2 billion represents lost productivity due to premature death.[1]

A comprehensive set of statistics regarding cancer incidence and mortality in the United States is complied each year by the National Cancer Institute; the report, called *Cancer Facts & Figures,* can be found at www.cancer.org. It is predicted that 1,444,920 new cases of cancer will be detected in the U.S. in 2007, and that 559,650 people will die as a result of cancer.[1]

Table 10.2 ranks the most commonly occurring cancers in men and women in the United States (excluding basal cell

U.S. Mortality, 2004			
Rank	**Cause of Death**	**No. of Deaths**	**% of Deaths**
1	Heart Disease	654,092	**27.2**
2	Cancer	550,270	**22.9**
3	Cerebrovascular diseases	150,147	**6.2**
4	Chronic lower respiratory diseases	123,884	**5.2**
5	Accidents (Unintentional injuries)	108,694	**4.5**
6	Diabetes mellitus	72,815	**3.0**
7	Alzheimer's disease	65,829	**2.7**
8	Influenza and pnuemonia	61,472	**2.6**
9	Nephritis	42,762	**1.7**

Table 10.1: Cancer is the second leading cause of death overall in the US, and the leading cause of death for people under 85 years of age [2].

Trends in Cancer Incidence and Mortality in the US:

From 1993-2002, cancer death rates in the United States dropped by 1.1% per year. The decrease in cancer mortality is attributed to a combination of better treatment, better early detection and cancer prevention. Death rates dropped more for men (1.5%/year than for women 0.8%/year). Cancer incidence rates in the United States have been stable since 1992 [4].

and squamous skin cancers). Prostate cancer is the most common cancer in US men, accounting for 1/3 of cancer incidence. Breast cancer is the most common cancer in US women, accounting for nearly 1/3 of cancer incidence; ovarian cancer, which we will study in detail later accounts for 3% of cancer incidence in US women.[3]

Table 10.3 ranks the most common causes of cancer mortality in men and women in the United States. In both sexes, lung cancer is the leading cause of cancer death, even though it is only the second most common cancer in men and women separately. As we will see later in this chapter, routine tests are available to screen older men and women for prostate cancer and breast cancer, so they tend to be diagnosed at an earlier, more curable stage. Because we do not have good screening tests for lung cancer, it tends to be diagnosed at a much later stage with worse prognosis. The situation is similar for ovarian cancer. Although it is responsible for only 3% of cancer incidence in women, it accounts for 6% of cancer mortality in women.[3]

The global burden of cancer

Globally, cancer is an important cause of mortality, accounting for 12% of all deaths worldwide (**Figure 10.2**). Cardiovascular disease is the leading cause of mortality worldwide, followed by infectious disease and cancer. Today, more than 11 million new cases of cancer are detected worldwide every year, and 6.7 million deaths can be attributed to cancer.[6] **Table 10.4** shows the leading causes of cancer mortality worldwide. In men, lung cancer is the most common cause of cancer death, while in women, breast cancer is the

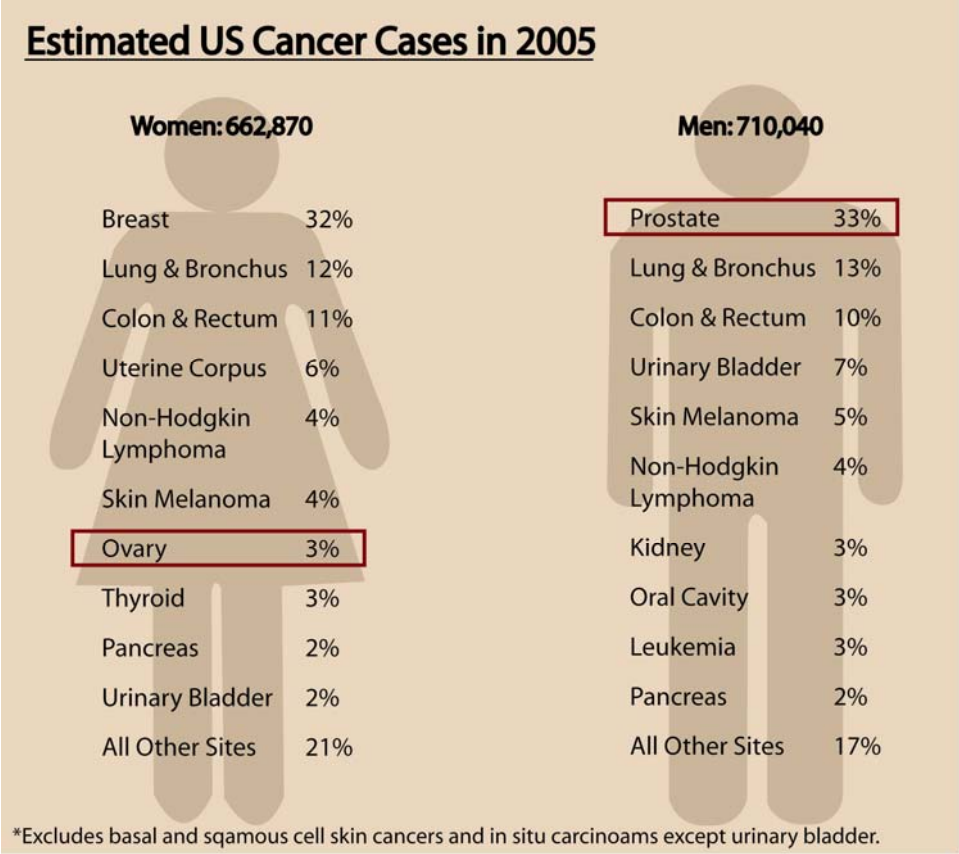


Table 10.2: The most commonly occurring cancers in men and women in the U.S. in 2005 [3]

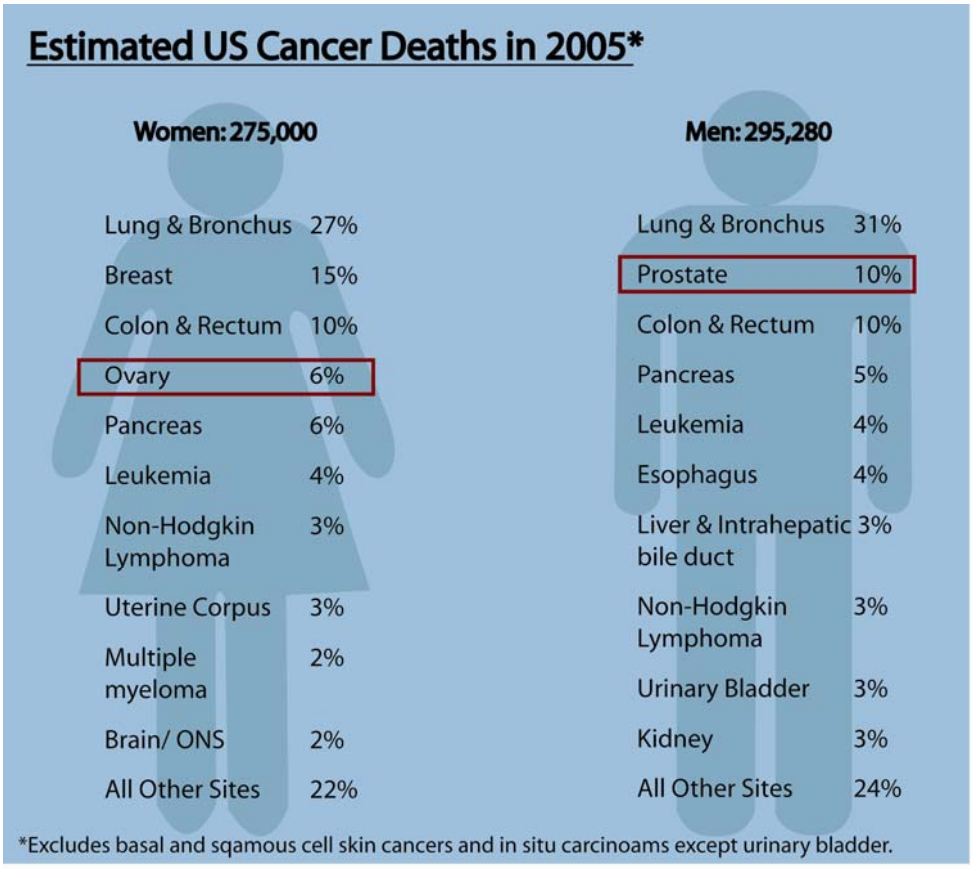


Table 10.3. The most common causes of cancer mortality in men and women in the U.S. in 2005 [3]

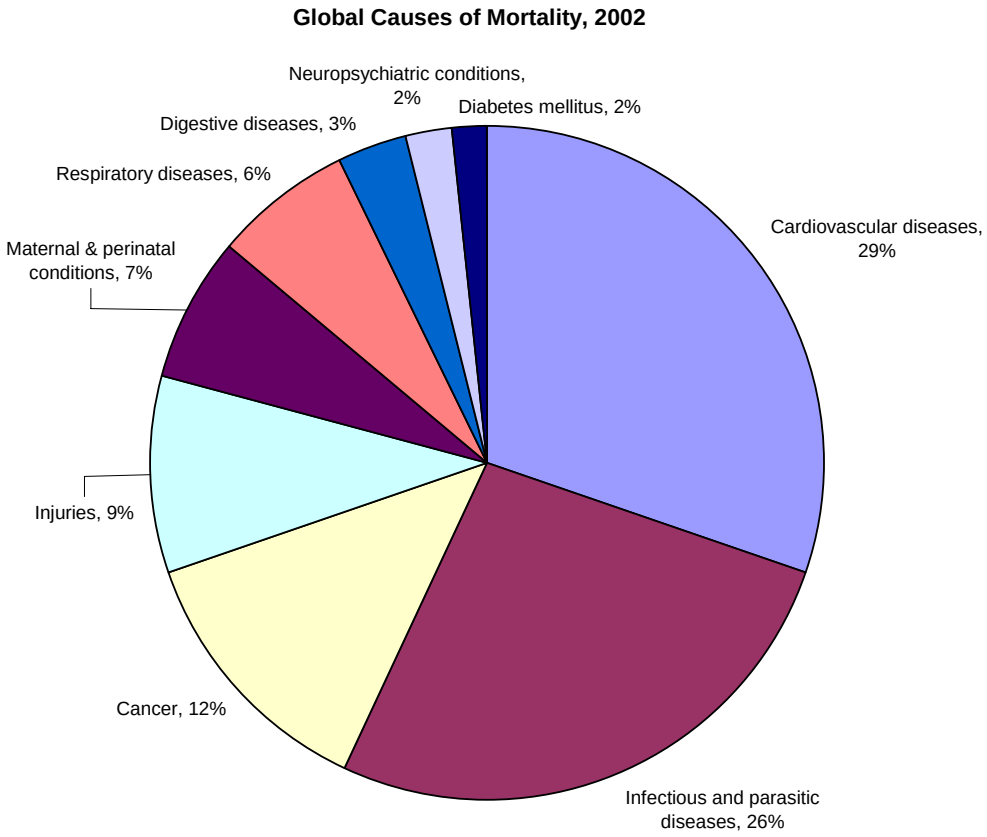


Figure 10.2: The most common causes of death worldwide in 2002. Cancer is the third leading cause of mortality, worldwide. Used with permission from [5].

most common cause of cancer death. The third most common cause of cancer death in women worldwide is cervical cancer; note that cervical cancer was not among the top ten

Table 10.4: The number of estimated cancer deaths worldwide in 2002 [6].

Estimated Worldwide Cancer Deaths in 2002*

Women: 2,927,896		Men: 3,795,991	
Breast	14%	Lung	22%
Lung	11%	Stomach	12%
Cervix uteri	9%	Liver	11%
Stomach	9%	Colon & Rectum	7%
Colon & Rectum	8%	Esophagus	7%
Liver	6%	Prostate	6%
Ovary	4%	Leukemia	3%
Esophagus	4%	Pancreas	3%
Pancreas	4%	Urinary Bladder	3%
Leukemia	3%	Non-Hodgkin Lymphoma	3%
All Other Sites	28%	All Other Sites	23%

*Excludes basal and sqamous cell skin cancers

causes of cancer incidence or mortality in the US. Again, as we will see later, this large difference can be attributed to the use of screening tools to detect cervical cancer at an early stage. In the developed world, the Papanicoloau (Pap) smear is used to screen the general female population for cervical cancer and its precursors. The early detection and treatment of these conditions prevents the development of invasive cervical cancer. Unfortunately, due to limited resources, cervical cancer screening is not implemented in many developing countries; as a result, cervical cancer is the leading cause of cancer death for women in developing countries.[6]

The maps in **Figure 10.3** illustrate global variations in the mortality of cancer today, and the changes predicted in cancer mortality throughout the world in the year 2020. Both the global incidence and mortality of cancer are predicted to increase. In the next 20 years, it is estimated that global cancer incidence will increase by nearly 50% and global cancer mortality will double. The largest rates of increase are predicted to occur in developing and newly industrialized countries. In 2020, more than 16 million new cancer cases are predicted, and 10.3 million people are expected to die of cancer.[7] Although the probability of being diagnosed with cancer is twice as high in developed countries, cancer survival rates are much lower in developing countries. In developed countries, about 50% of cancer patients die as a result of their cancer, while in developing countries more than 80% of cancer patients already have late-stage incurable tumors at the time of their diagnosis.[8] It is estimated that by 2020, at least 70% of cancer deaths will occur in developing countries, where resources for early detection and treatment are least available.[9]

If you live in the US, what is your lifetime risk of developing cancer? If you are female, you have a 33% chance of developing cancer at some time in your life, with a 14% chance of developing breast cancer at some point in your life (**Table 10.5**). If you are male, you have a 50% chance of developing cancer at sometime in your life, with nearly a 17% chance of developing prostate cancer at some point (**Table 10.6**).[10]

How can you reduce your cancer risk? More than 1/3 of cancers are preventable, through three approaches: (1) reducing tobacco use, (2) implementing existing screening techniques worldwide, and (3) adopting a healthier lifestyle and diet. Globally, 43% of cancer deaths are due to tobacco use, inappropriate diet or infection.[7] In developing countries, infectious agents are responsible for nearly 25% of

Site	Risk
All Sites	1 in 3
Breast	1 in 8
Lung and bronchus	1 in 17
Colon and rectum	1 in 18
Uterine Corpus	1 in 38
Non-Hodgkin lymphoma	1 in 55
Ovary	1 in 68
Melanoma	1 in 77
Pancreas	1 in 79
Urinary bladder	1 in 88
Uterine cervix	1 in 135

Table 10.5: Risk for **women** of developing cancer over the course of a lifetime.

Site	Risk
All Sites	1 in 2
Prostate	1 in 6
Lung and bronchus	1 in 13
Colon and rectum	1 in 17
Urinary bladder	1 in 28
Non-Hodgkin lymphoma	1 in 46
Melanoma	1 in 52
Kidney	1 in 64
Leukemia	1 in 67
Oral Cavity	1 in 72
Stomach	1 in 82

Table 10.6: Risk for **men** of developing cancer over the course of a lifetime.

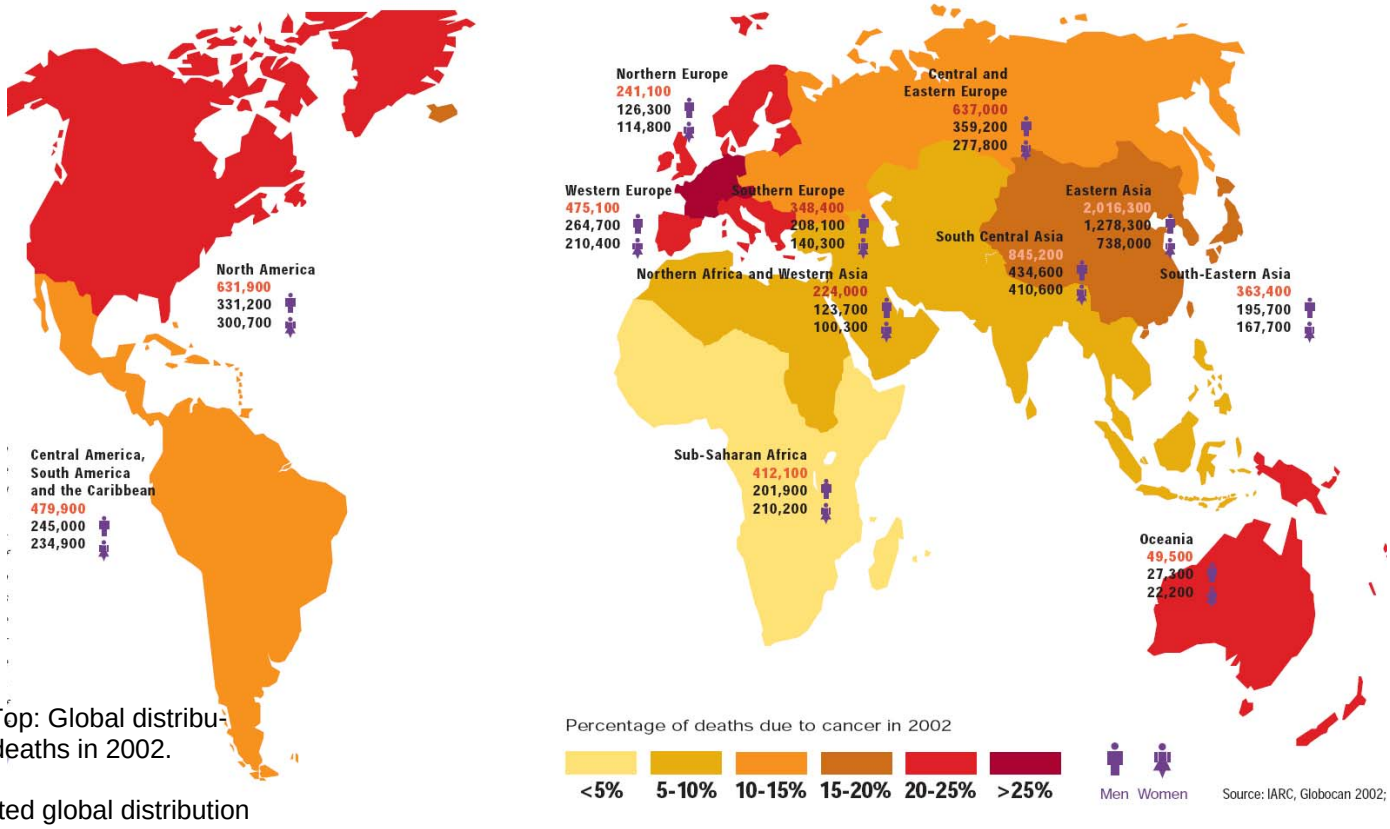
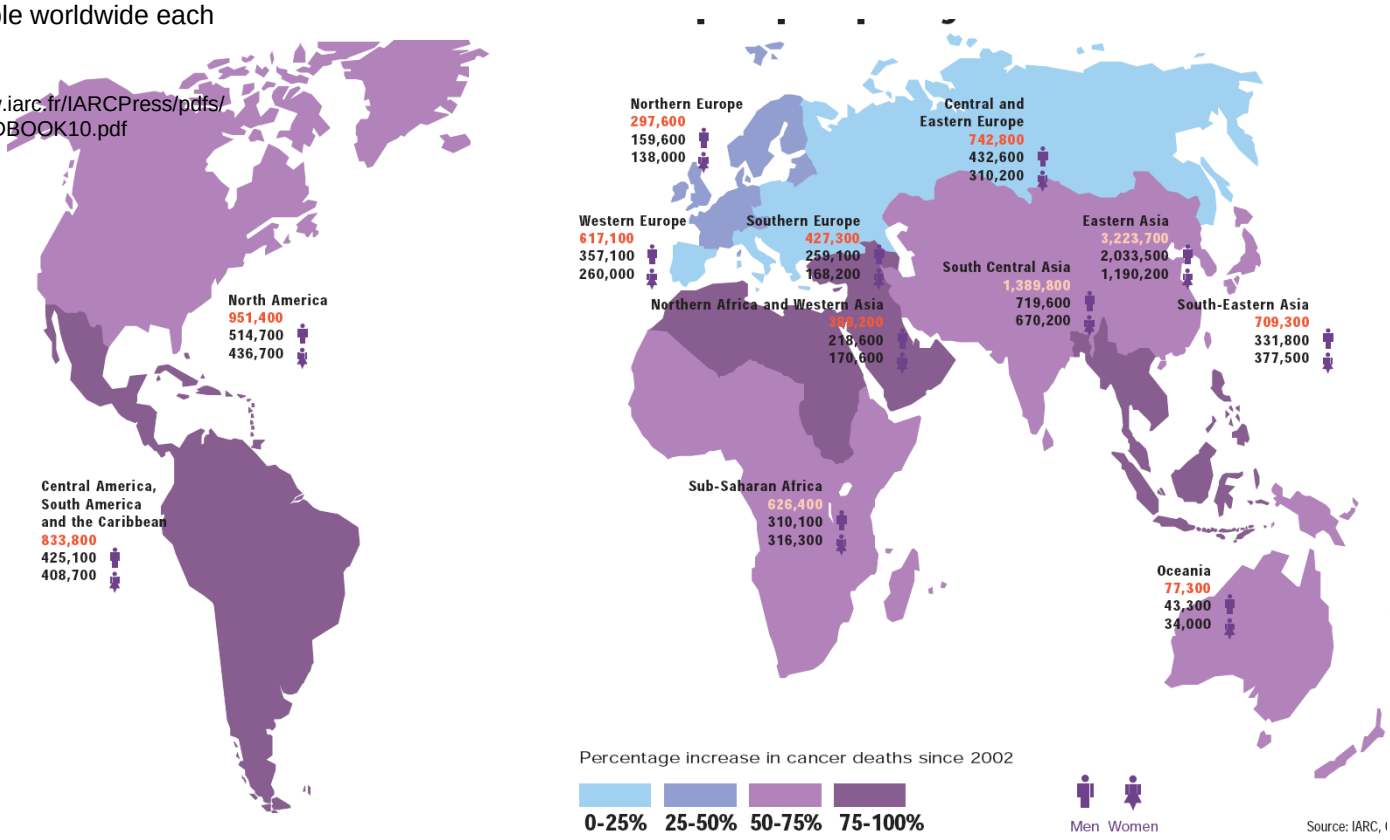


Figure 10.3: Top: Global distribution of cancer deaths in 2002.

Bottom: Predicted global distribution of cancer deaths in 2020. By 2020, cancer is predicted to kill more than 10 million people worldwide each year.

Source: http://www.iarc.fr/IARCPress/pdfs/handbook10/HANDBOOK10.pdf



cancers, while only 9% of cancers are due to infectious agents in developed countries. These include hepatitis B and C (can lead to liver cancer), the sexually transmitted human papilomavirus (HPV) (can lead to cervical cancer), and Helicobacter pylori (can lead to stomach cancer). As we will see later, vaccination may be the key to preventing these cancers.[11]

Figure 10.4 shows the relationship between per capita cigarette consumption and lung cancer rates in men and women.[12] There is a 20-25 year delay between the peak in cigarette consumption and the peak in lung cancer incidence, reflecting the long period of exposure and resulting biological changes which occur in lung cancer. Rates of lung cancer incidence in women peak about a decade later than in men, reflecting the delay in when women began to smoke. While tobacco use has declined in many developed countries, it is rising in many developing countries. Worldwide about 35% of men in developed countries smoke, while the fraction of men who smoke is 50% in developing countries. China represents a particular concern; with more than 300 million male smokers today, future increases in lung cancer incidence and mortality are a likely consequence.[13]

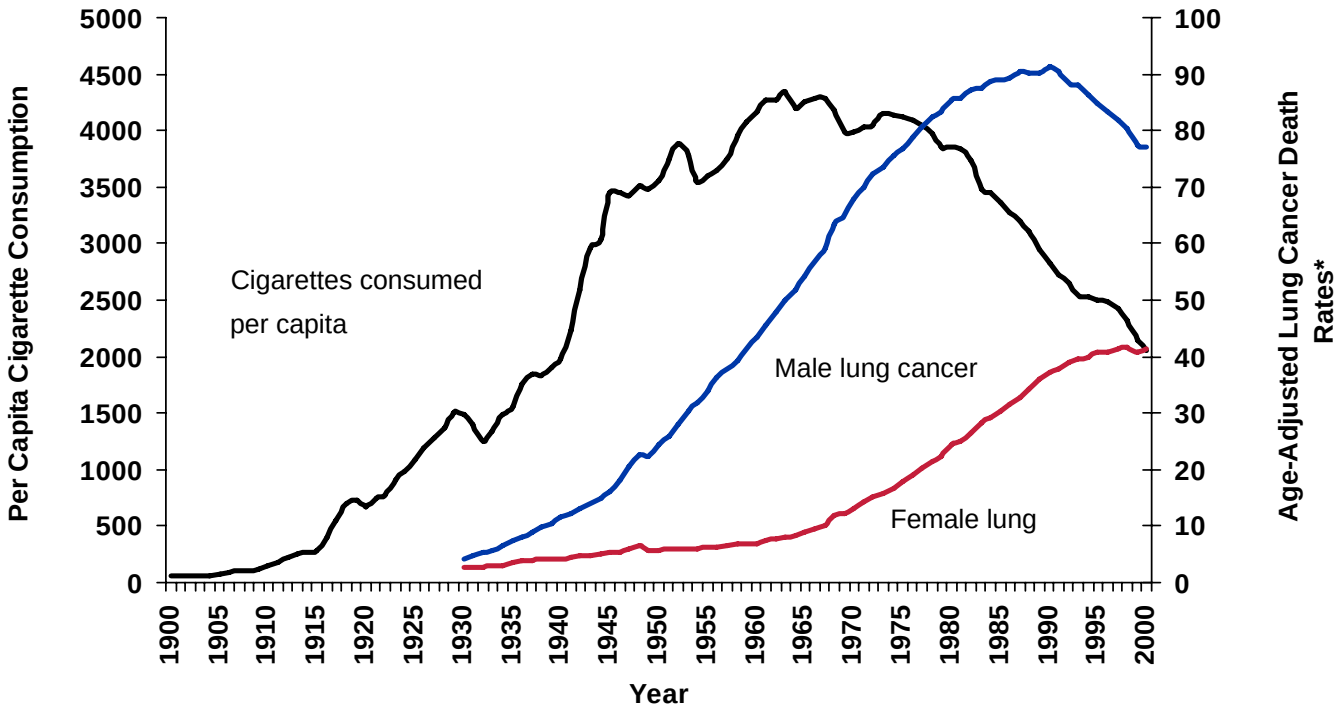
Changes in diet can also reduce cancer risk. The American Cancer Society recommends that persons consume five serv-

Figure 10.4: An increase in the mortality due to lung cancer in the US followed an increase in cigarette consumption that begin in the 1930s.

Source: Death rates: US Mortality Public Use Tapes, 1960-2000, US Mortality Volumes, 1930-1959, National Center for Health Statistics, Centers for Disease Control and Prevention, 2002. Cigarette consumption: US Department of Agriculture, 1900-2000.

http://www.cancer.org/downloads/STT/**Cancer_Statistics_2005_Presentation.ppt**

Tobacco Use in the US, 1900-2000



***Age-adjusted to 2000 US standard population.**

ings of fruits and vegetables daily to reduce their cancer risk. Unfortunately, less than ¼ of Americans follow this recommendation; only 24.3% eat 5 or more servings of fruit and vegetables daily, a figure that has changed little over the last decade. [14]

Part I: Peace Corps and Rice Visits: July 2 - July 4, 2007

Tessa **Swaziland**

I got up around the same time as I usually do for clinic (6:45-ish) to shower and finish packing for my long awaited visit to a Peace Corps volunteer's site. Carrie had suggested I stay with a volunteer as soon as I got here, but once WFP stuff started, I didn't have any time to escape the clinic. We both thought it would be a good opportunity to see where the COE's patients come from—not just physically, but culturally, emotionally, etc…Like, what kind of customs and beliefs exist in their communities? How are decisions made? What is the family environment like? How are orphans and abandoned children cared for? What is the system for governing? And also, this would give me an opportunity to talk with someone whose work and goals were similar to mine but who had been here much longer and worked on many more projects.

Carrie gave me a volunteer's number a couple weeks ago, and I had called her and set up a time to come. Tandi (that's the volunteer's Seswati name) and I met downtown by an Internet café. She was very nice and happy to answer all of my questions. In what ended up being a very rushed morning (there had been some confusion with the new WFP system, and Dave called me to the clinic mid-shower) I hadn't managed to squeeze in breakfast, so we sat down for omelets and coffee before heading out to her homestead. I was certainly glad for her company. I'd been on combies before (the vans used for public transport here), but only for short trips between the clinic and Mbabane. Finding my way from Mbabane to Manzini, switching to another combie, riding from Manzini to Siphoneni, transferring to yet another combie, and riding out to her village (name was hard to pronounce, and now I've forgotten it completely) would've been quite an adventure (possibly an unsuccessful one).

When we got off the last combie, we walked by a row of gogos (old women selling fruit or other items on the side of the road) and she greeted all of them with "Sanibonani" (the "hello" you use to address a group of people). A chorus of "Yebo"s echoed back, and the exchange continued for a minute. Everyone we passed, Tandi greeted and waved to. In that particular community, they used a two hand wave, which proved difficult since we were both carrying quite a bit of stuff. Discovering I had no Seswati name, Tandi enlisted the help of the gogos in naming me. I am now officially Zandile Dlamini. "Zandile" means "too many girls," and Dlamini is the most common last name in Swaziland. I'd say about 40% of our COE patients are Dlamini's.

We hiked for about 20 minutes to reach the homestead where she had been living for almost a year. The paths were dirt trails randomly winding and crisscrossing through brush and occasionally along pastures and homesteads. It reminded me quite a bit of the landscape and random layout of the community I lived in 5 years ago in Nicaragua.

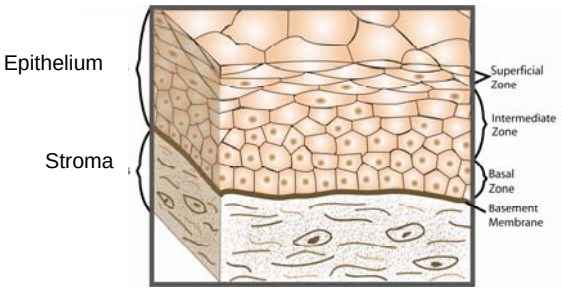
At her home, I met her gogo (literally translate as "grandmother") and the children who lived there. Her babe (father of the house)

wasn't home. In fact, he rarely is there, she said. Two of his three wives reside at that particular homestead, but neither was there at the time. One was shucking corn on the other side of the nearby mountain, and I'm not sure where the other one was. The one shucking was Tandi's "mage" (mother… pronounced ma-ge with a soft g sound), and I got to meet her later. She was very well educated and easy to talk to. In fact, she had met her husband when they were both studying at a university in the UK. At that time he already had two wives back in Swaziland. She had hosted Peace Corps volunteers several times before and seemed like a very good host mom. She kept trying to make Tandi stop translating. (She thought I was another PC volunteer and thought I should know the language by now.) She said all the children there were her own, but Tandi told me later that in fact, they were all children of her husband, but none actually belonged to her. They were the children of all of his girlfriends. (Keep in mind; this is a completely normal arrangement for a family. Other than the fact that they were wealthy for a rural Swazi family, this was very representative of many of the homesteads all over Swaziland. A homestead is basically a collection of homes that belong to one extended family—a gogo, a babe, several mages, and many children).

Anyway, after I met the gogo (and before I met the mage) Tandi took me over to the hospital and VCT (voluntary counseling and testing facility) to see what they were like. It looked much like the Vuvulane clinic except bigger and cleaner (still nothing close to the COE standards). We talked to the VCT employees for a bit. They basically serve as a site where community members can come in and get tested for HIV. If they test positive, there are support groups and counseling available. Also, if they've been diagnosed and prescribed ART, they can pick up the meds there. One nice thing about this particular VCT was the fact that there was a woman who worked there whose sole responsibility was to deal with adherence. She was Swazi, and I believe she actually grew up there or nearby. She had obtained a grant for her project and was now trying to improve adherence in the community. Unfortunately, she had left for the day, so I didn't get to speak with her.

On the way back to the homestead, Tandi told me more about her experiences living in Swaziland. She said adjusting to the culture wasn't too difficult. She didn't really get homesick, and the community welcomed her. Apparently the last volunteer was kind of angry. Whenever Tandi had events, people would always come up to her afterwards and say, "Thank you for not yelling at me, Sisi." (Sisi means sister and is the word everyone uses to address a young woman who isn't married). Evidently, the last volunteer had been a yeller. The one thing Tandi said was most difficult to adjust to was the number of deaths. Every week, there are about three vigils to mourn the deceased. They last all night and end at about 7 a.m. with a funeral.

Figure 10.5: Cartoon of squamous epithelium. Multiple layers of epithelial cells sit atop a basement membrane. Cells become progressively more differentiated as you move from the basement membrane toward the epithelial surface.



The Pathophysiology of Cancer

Now that we have examined the global burden of cancer, let's turn to how cancers develop. While the growth and differentiation of normal cells is carefully coordinated by growth signals, cancers are characterized by uncontrolled growth and spread of abnormal cells. Unlike normal cells, cancer cells continue to grow in the absence of external growth signals, and they ignore signals to stop dividing, to specialize or to die.[15] Cancer cells can not only sustain themselves, they can expand and migrate. Normal cells can be transformed into cancer cells by a number of factors, including exposure to external carcinogens such as tobacco smoke, certain chemicals, or ionizing radiation, exposure to certain infectious agents, and exposure to certain hormones.

At the cellular level, the process of cancer development is remarkably similar in many different tissues. More than 85% of cancers arise in the epithelial tissues that line our organs, such as the skin, the digestive tract, the respiratory tract, and the genitourinary tract. **Figure 10.5** shows a cartoon of one type of epithelial lining. In many tissues, the epithelial surface consists of multiple layers of epithelial cells. These cells sit on top of a special membrane called the basement membrane. Beneath the basement membrane there are layers of muscle and connective tissue that give the organ its structural stability. The epithelium is exposed to the external world; it serves as an important protective barrier, and is constantly regenerating itself. The cells adjacent to the basement membrane are responsible for this regeneration, therefore they are the most metabolically active cells in the epithelium. Epithelial cells become more specialized and mature as we move closer to the surface of the epithelium. Those cells at the top of the epithelium are dead, and will eventually be sloughed off.

Thus, there is a gradient of cell differentiation throughout the epithelium with the most differentiated cells at the epithelial surface and the least differentiated cells at the basement membrane, and the morphology of normal cells differs along this gradient. As we move from the bottom to the surface of the normal epithelium, the nucleus of each cell occupies progressively less and less of the cell volume. This ratio is called the nuclear to cytoplasmic ratio (N/C ratio). A large N/C ratio is characteristic of a rapidly dividing immature cell, while a small N/C ratio is characteristic of a mature, terminally differentiated cell. Figure 10.6 shows a photograph of a biopsy from the oral mucosa that has been sectioned transversely and stained with hematoxylin and eosin dyes; the hematoxylin colors the nucleus purple. The cells adjacent to

the basement membrane have a large N/C ratio and this ratio becomes progressively smaller as we move up through the normal epithelium.

An epithelial cancer begins with transformation of a single epithelial cell. As this transformed cell grows, it fails to differentiate, continuing to actively divide. When the lower 1/3 of the epithelium is filled with transformed cells, the condition is known as low grade precancer. When the lower 2/3 of the epithelium is occupied by transformed cells, the condition is known as high grade precancer. **Figure 10.7** shows a photograph of an oral mucosa biopsy with a high grade precancer on the right. Note the cells with increased N/C ratio in the lower 2/3 of the epithelium. These lesions are called pre-cancerous lesions because they are not yet cancers, but they have the potential to develop into a cancer. As shown on the left of **Figure 10.7**, in some cases transformed cells can break through the basement membrane, entering the stroma beneath. In this case, we no longer have an organized epithelium and stroma; instead the tissue is a mix of nests of cancer cells and surrounding stroma. This is a significant phenomenon – it is at this point that we go from precancer to invasive cancer**,** and as we will later see, the prognosis and treatment of precancer and cancer are radically different.

How do epithelial cells become transformed to initiate the development of a cancer? A cell is transformed through a series of mutations that affect its DNA; DNA mutation leads to production of mutant proteins. For example, a mutated gene can produce a defective protein that causes the growth factor receptors on the cell's surface to be constantly on. This type of ''gain of function' mutation can produce a transformed cell which continues to grow in the absence of

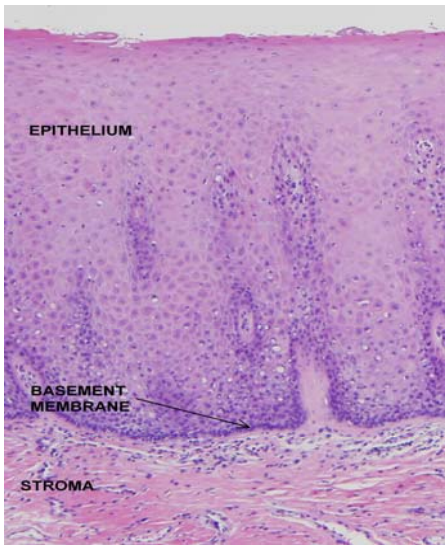


Figure 10.6: Photo of stained biopsy of the oral mucosa. Multiple layers of epithelial cells sit atop a basement membrane. Cells become progressively more differentiated as you move from the basement membrane toward the epithelial surface.

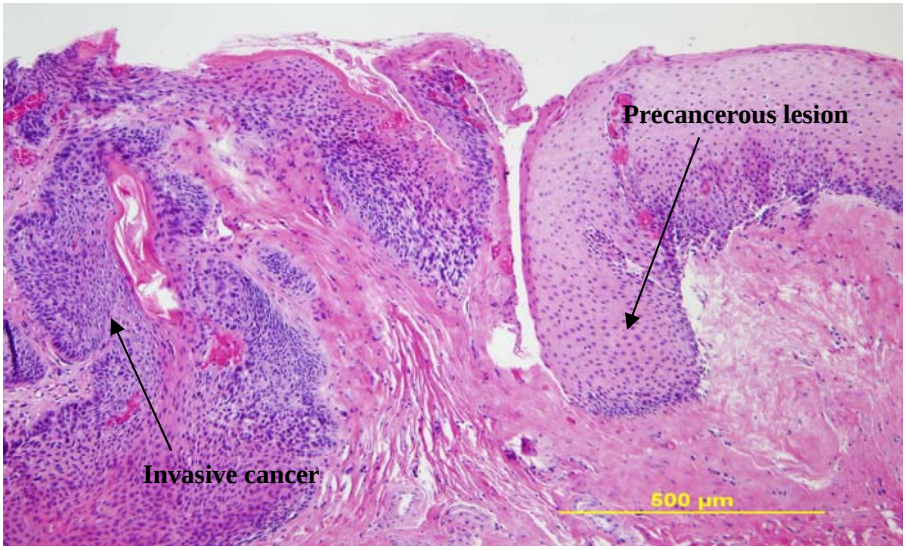


Figure 10.7: Photo of stained biopsy of the oral mucosa. On the right side of the biopsy, a high grade precancerous lesion is present. Note the cells with large N/C ratio that occupy most of the epithelium. The left side of the biopsy shows an invasive cancer of the oral mucosa. Nests of cancer cells are intermixed with stromal tissue.

Read More About It:
A comprehensive overview of cancer biology can be found at **www.insidecancer.org**. The section "Pathways to Cancer" contains 3D animation that illustrate the abnormalities in signaling pathways associated with cancer cells.

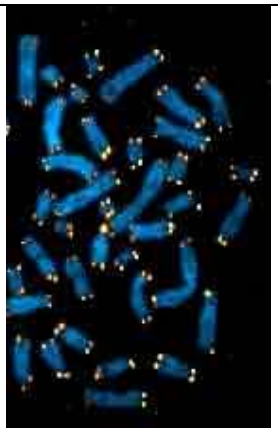


Figure 10.8: A fluorescent stain indicates telomeric DNA (yellow) at the tips of chromosomes.
http://www.bccrc.ca/tfl/people_plansdor.html

external signals. The DNA in cancer cells can also undergo mutations which result in a loss of function; for example, ignoring signals to stop growth, or losing the function to repair or destroy defective cells.

Thus in cancer, oncogenic mutations disrupt the cell cycle and the careful coordination of growth, differentiation and death that characterizes normal cells and tissues. Cancer cells don't respond to signals that regulate cell growth and division. They can grow in the absence of signals to grow, and they ignore signals to stop growth. Changes in the gene expression profile allow cancer cells to replicate indefinitely. For example, normal cells can divide a finite number of times. Cancer cells overcome this limitation to become immortalized. Normal cell division is regulated by telomeric DNA at chromosome ends (**Figure 10.8**). This DNA functions to prevent end to end fusion of chromosomes. Normal cells shorten telomeric DNA with each division. After a certain point telomeres fuse and cells die. Most cancer cells activate an enzyme called telomerase. This enzyme extends the telomeres so that the cell can go through unlimited cycles of cell division.[16]

The mutations that lead to cancer start in one cell, and as

this cell divides, further mutations can occur in daughter cells. It is the accumulation of mutations that irreversibly transforms a normal cell into a cancer cell. Usually 5-7 mutations are required to transform a cell. These mutations accumulate over time, which is why cancer is more common with increasing age. Fewer than 10% of mutations that lead to cancer are inherited; most are due to environmental factors.[16]

We can think of tumor development as analogous to Darwinian evolution. The transformation of a normal cell into a cancer cell occurs via a succession of genetic changes; together, these changes lead to the progressive conversion of normal cells to cancer cells.[15] Because these changes confer a growth advantage relative to normal tissue, the unchecked growth of cancer cells results in a mass of tumor cells. Once a nest of transformed cells begins to grow, the energy demands of these cells rapidly outstrip the capacity of the normal vasculature to supply nutrients.[16] As a result, transformed cells can induce the formation of new blood vessels, in a process called angiogenesis (**Figure 10.9**). Angiogenesis can occur in the early precancerous stages. Frequently, the vessels formed in a tumor are abnormal—they are tortuous and leaky; we will see later that we can exploit these properties to aid in the early detection and treatment of tumors.

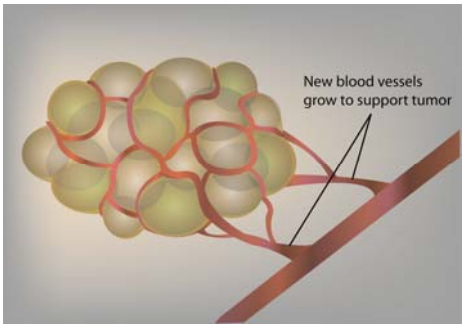
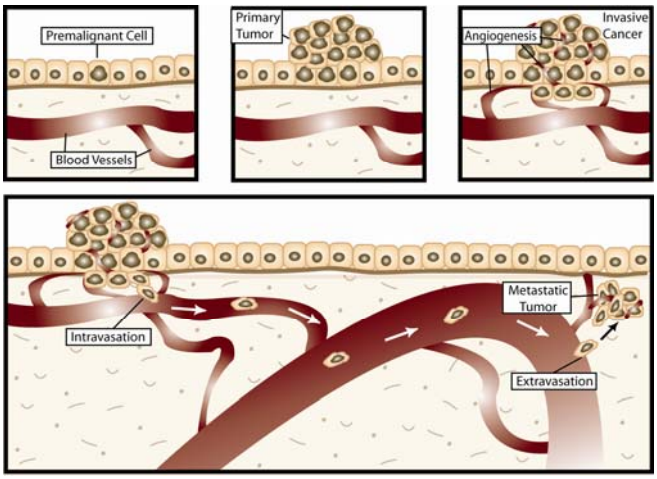


Figure 10.9: During angiogenesis, tumor cells induce formation of new blood vessels.

When cancer cells are confined to the organ in which they originated, we refer to the lesion as a primary tumor. Cancer cells have the ability to spread beyond the primary organ site (**Figure 10.10**). As cancer cells invade through the connective tissue in the primary organ site, they can intravasate into blood vessels and lymph vessels in that organ. From there, they can travel to distant organs and extravasate out of the blood vessels to form metastatic nests of tumor cells in distant organs.[17] Some of these nests of tumor cells will survive, grow and expand to form metastatic tumors. **Figure 10.10** illustrates how a single transformed cell can lead to the development of a metastatic tumor. Metastasis is responsible for a large fraction (90%) of death due to cancer.[16]

Figure 10.10: Development of a metastatic tumor.



In summary, there are more than 100 different types of cancer, yet the process of tumor development is remarkably similar across different organ sites. The formation of tumors is a multi-step process, during which six essential alterations occur in cell physiology: (1) cells develop self-sufficiency in growth signals, (2)

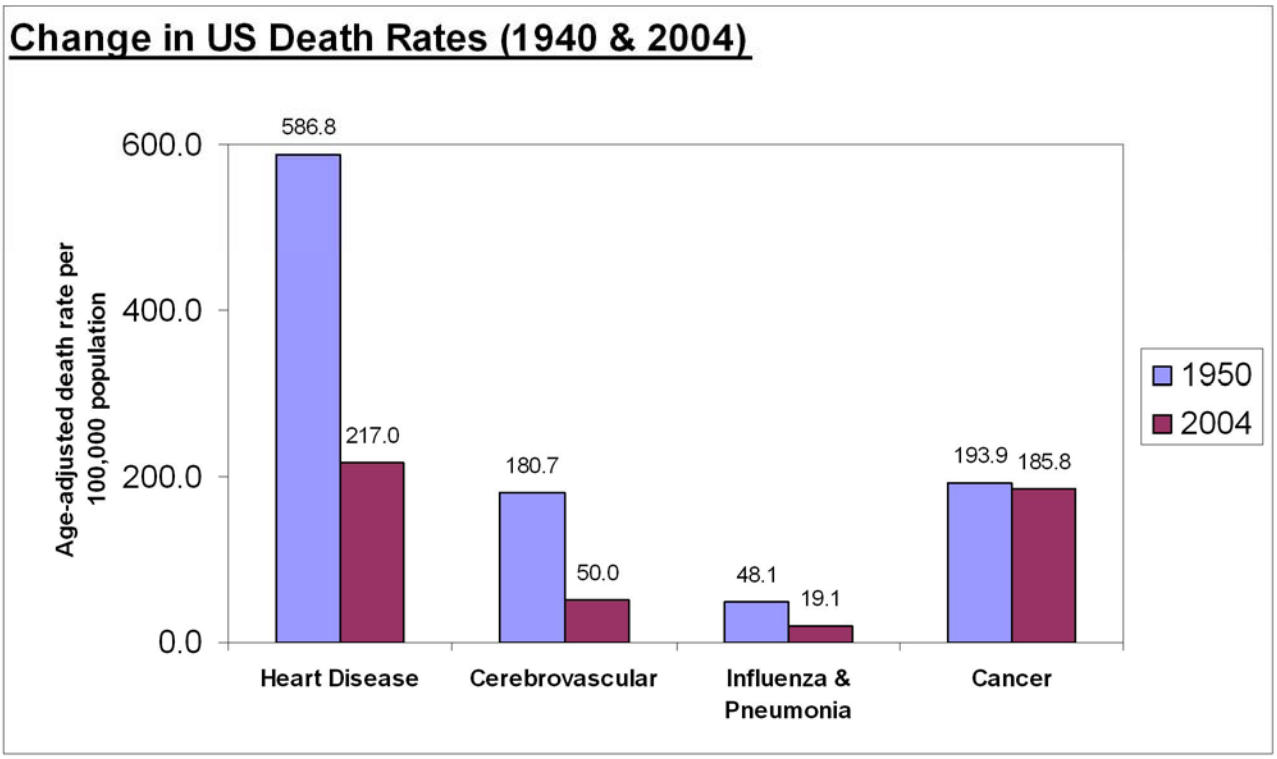


Figure 10.11: Changes in U.S. death rates from 1950 to 2004.

they become insensitive to signals of growth inhabitation, (3) they evade programmed cell death, (4) develop limitless replicative potential (5) they can sustain angiogenesis, and (6) acquire the ability to invade tissue and metastasize.[15] As we will see later in this chapter, the development of new diagnostic and therapeutic techniques for cancer increasingly focuses on these six common elements of cancer cells.

Site	1975-1977	1984-1986	1996-2002
All Sites	50%	53%	66%
Breast (female)	75%	79%	89%
Colon and rectum	51%	59%	65%
Leukemia	35%	42%	49%
Lung and bronchus	13%	13%	16%
Melanoma	82%	86%	92%
Non-Hodgkins lymphoma	48%	53%	63%
Ovary	37%	40%	55%
Pancreas	2%	3%	5%
Prostate	69%	76%	100%
Urinary Bladder	73%	78%	82%

Table 10.7: Changes over time in 5-year survival rates.[1]

Why is early detection so important?

In his 1971 State of Union address, President Nixon declared “war” on cancer and requested $100 million for cancer research. On December 23, 1971, Nixon signed the National Cancer Act into law and said, "I hope in years ahead we will look back on this action today as the most significant action taken during my Administration."[18] Today, the US government still makes a substantial investment in cancer research; the National Cancer Institute (NCI) will spend over $4.6 billion for cancer research in 2007.[19] The mission of the NCI is to eliminate

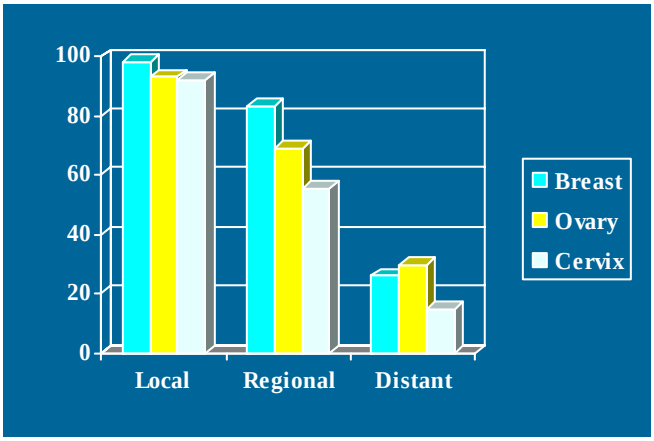


Figure 10.12: Five-year relative survival rates due to several different cancers.

suffering and death due to cancer by 2015.[20]

How have cancer incidence and mortality rates changed as a result? In 1950, heart disease was the leading cause of death, followed by cancer, cerebrovascular disease, and infectious disease (**Figure 10.11**). More than 50 years later, in 2004, the age adjusted mortality due to heart disease, cerebrovascular disease, and infectious disease have all dropped by more than half; that due to cancer has decreased only slightly.[21] **Table 10.7** shows the 5-year survival rates for patients diagnosed with different cancers dur-

Cancer Pain Management: China

Cancer pain: it is a component of the disease that many patients fear more than death itself. Its severity has been described as intolerable and excruciating, and it only increases with the progression of the cancer. While cancer pain is treatable with the use of standard analgesics, including opioids such as morphine, this cheap and effective analgesic is not the standard of care everywhere.

"It was clear that many places didn't even have aspirin for cancer pain relief. And in many countries, the idea of having more potent drugs, even codeine let alone morphine, which is what we use for the managing of severe cancer pain, was nonexistent," recalls Dr. Charles Cleeland, Chair of the Department of Symptom Research at the University of Texas M.D. Anderson Cancer Center.

In 1993 the morphine consumption in China was estimated at 0.01 mg per capita, a surprisingly low number compared to the estimated 66.53 mg per capita consumption for Denmark [22]. Morphine was strictly managed. Suffering Chinese patients needed a certificate to receive morphine, and could only receive one ampoule of short-lasting morphine per day for four days as long as they returned an empty ampoule every time [22]. Patients had to choose the hour of their pain relief. Such telling statistics urged the World Health Organization (WHO) to initiate a plan to improve cancer pain management globally.

Up to this point, Dr. Cleeland, whose work focuses on pain assessment and treatment, had worked in countries such as Mexico and the Dominican Republic, instructing small groups in the usage of opioids for cancer pain relief. "At that time I had a postdoctoral fellow from China who said, 'Why don't you take on something really big?' So she got me connected with a friend of hers who was the minister of the health for the district of Beijing. So he came over and we tried to think of things to do to start."
Thus began a collaboration between the WHO, the government of China and Dr. Cleeland's pain research group to address the inadequate cancer pain management in China. The initial step was to study the epidemiology of the problem. To do this Dr. Cleeland launched a study led by Dr. Shelley

Wang in 1992. The study helped elucidate the current state of pain control in the greater area of Beijing by examining 200 different cases. "And what we found was not a surprise; morphine was to be used rarely if at all. These patients had very high levels of pain compared to more developed countries."

To try and explain the kind of pain the group encountered, Dr. Cleeland says, "On a 0 to 10 scale, if we break that scale up you and I probably experience from time to time pain maybe up to 3 or 4. Many cancer patients after their disease has metastasized have a continual 10 pain."

The lack of pain control was evident, and the reasons behind this were many. "There were regulatory problems. There were issues of concern with addiction and a lack of any kind of distribution of controlled substances. It was a multifaceted problem."

The next step was to set up large meetings in Beijing and other major cities to introduce the epidemiology of the problem and to begin instruction. Over 500 individuals selected by government officials attended these three-day meetings. "They chose very well. They picked people who were administrators for major hospitals. They picked drug regulators. They picked nursing people as well as physicians."

Once the problem was discussed, a series of "trainer training" programs began. Groups composed of a nurse, a pharmacist and a physician from different hospitals would attend the program and learn about pharmacology of opioids and pain assessment. "The impressive thing, always, was when we'd train the students and then send them into the wards. They would ask the physicians how many patients have pain and the physicians would say "Well, of course, none of my patients have pain." Then they would go around and ask and find it was quite a different story."

Gradually, the training was handed over to Chinese professionals. To support their work, an evidence-based text developed by the WHO in the subject of cancer pain treatment was translated to Chinese. Concomitantly, the government of China adopted a new cancer pain relief policy, adjusting the inhibiting narcotics control policy, approving new opioid analgesics for sale and distribution, and increasing opioid manufacturing through joint ventures and other means. Gradually the pain alleviation for suffering cancer patients spread throughout the country.

Five years later, a comparison study was done. "It was a tremendous change from the majority of patients being under-managed to a majority of patients being managed very well according to the WHO standards. It was a 50% drop, from 60% under-managed in 1992 to 30% under-managed in 1997," Dr. Shelley Wang recalls.

Today opioids such as immediate and sustained-release morphine, fentanyl patch, meperidine, methadone and codeine are available to cancer patients in China. Physicians can prescribe these analgesics for up to 5 days, and in the case of more severe pain a physician can prescribe 15-day relief in the form of the fentanyl patch [23]. And, although the work is still not complete, a network of committed policy makers, oncologists and pain experts have been able to tremendously change the excruciating pain experience for a nation.

Unfortunately, the beginning of this story is not uncommon. "You have about 10 million cancer deaths a year worldwide, and probably 2/3 of them will experience significant pain. And, unfortunately, at least more than half will be inadequately managed. So they'll have a period of 3 or 4 months at the end of life which will be just miserable," states Dr. Cleeland.

Presently, Dr. Cleeland, Dr. Wang and their group continue to address the cross-cultural issue of pain and agree that its poor treatment is due to the lack of appreciation and assessment of pain. They hope to reproduce the successful model established in China and are involved in joint efforts to help other nations such as Russia, Korea and Japan, bring relief to cancer patients.

Sources [22, 23].

Screening Guidelines for the Early Detection of Breast Cancer, American Cancer Society

- Yearly mammograms are recommended starting at age 40 and continuing for as long as a woman is in good health.
- A clinical breast exam should be part of a periodic health exam, about every three years for women in their 20s and 30s, and every year for women 40 and older.
- Women should know how their breast normally feel and report any breast changes promptly to their health care providers. Breast self-exam is an option for women starting in their 20s.
- Women at increased risk (e.g., family history, genetic tendency, past breast cancer) should talk with their doctors about the benefits and limitations of starting mammography screening earlier, having additional tests (i.e., breast ultrasound and MRI), or having more frequent exams.

Table 10.8: Recommendations for breast cancer screening from the American Cancer Society [1].

ing three different time periods: 1975-1977, 1984-1986 and 1996-2002. In the mid 1970s, the overall 5-year survival rate was 50% and this did not change appreciably over the next decade. In the late 1990s to early 2000's, the 5-year survival rates had risen to 66%. Prostate cancer showed great improvement between the 1980s and 1990s, reflecting the introduction of a screening test designed to detect early disease.[1] Lung cancer and pancreatic cancer survival rates have not changed substantially during this period; these cancers tend to be diagnosed at a relatively advanced stage when available therapies are not particularly effective.

Why is early detection so important to reducing cancer mortality? **Figure 10.12** compares the 5-year survival rates for three different cancers as a function of the stage at which they are diagnosed. The 5-year survival rates exceed 90% for patients whose cancer is diagnosed when it is still confined to the local organ site.[1] The 5-year survival rate drops when cancer is first detected after metastasis to a regional location. For those patients whose cancer has metastasized to a distant location prior to diagnosis, the 5-year survival rates are dismally low. Thus, an important strategy to reduce the mortality associated with cancer is to develop improved detection technologies designed to identify cancer at the earliest possible stages, when the available therapies are more likely to result in cure.

Strategies for early detection

Typically, cancers do not produce symptoms until a fairly late stage. How can we identify disease in asymptomatic patients? This is the goal of a process called 'cancer screening'. Screening refers to the use of simple tests in a healthy population. The goal of screening is to identify individuals who have disease, but do not yet have symptoms. The goal of screening is not to diagnose disease, but rather to use an inexpensive and simple test to identify those individuals who should have a more expensive and accurate test to confirm a diagnosis of disease.

Screening Guidelines for the Early Detection of Colorectal Cancer, American Cancer Society 2003

- Beginning at age 50, men and women should follow one of the following examination schedules:
  - A fecal occult blood test (FOBT) every year.
  - A flexible sigmoidoscopy (FSIG) every five years.
  - Annual fecal occult blood test and flexible sigmoidoscopy every five years*.
  - A double-contrast barium enema every five years.
  - A colonoscopy every ten years.

*Combined testing is preferred over either annual FOBT, or FSIG every 5 years alone. People who are at moderate or high risk for colorectal cancer should talk with a doctor about a different testing schedule.

Table 10.9: Recommendations for screening for cancers of the colon and rectum from the American Cancer Society [1].

	Mammogram Positive	Mammogram Negative
Patient Has Breast cancer	True Positive (TP)	False Negative (FN)
Patient Does Not Have Breast Cancer	False Positive (FP)	True Negative (TN)

Figure 10.13: Possible outcomes of a screening mammogram for breast cancer.

Table 10.10: Comparing the effectiveness of a breast exam versus a mammogram in screening for breast cancer [25].

Accuracy of Breast Cancer Screening		
	Sensitivity	Specificity
Clinical breast exam	54%	94%
Mammography	75%	92%

In the US, we routinely screen for four cancers, including female breast cancer with clinical breast examination and screening mammography, cervical cancer with HPV testing and the Pap smear, prostate cancer using the serum PSA test and digital rectal examination, and colon and rectal cancer, using a combination of the fecal occult blood test, flexible sigmoidoscopy, and colonoscopy.[24]

Table 10.8 summarizes the recommendations of the American Cancer Society regarding screening for breast cancer; yearly mammograms to screen for breast cancer are recommended for women over the age of 40. While 69% of women over age 40 report having received a mammogram, women with no health insurance are significantly less likely to have received a mammogram. **Table 10.9** summarizes the recommendations of the American Cancer Society regarding screening for cancers of the colon and rectum. While the percentage of people age 50 or more reporting a recent flexible sigmoidoscopy has increased in the last few years, only 45% of patients have had this test. The percentage of patients with no health insurance reporting this screening test is only 17%.[14]

Effectiveness of Screening:

How do we judge the effectiveness of a screening test? Let's take the example of screening for breast cancer. Imagine that you are a patient being screened with mammography. We can envision four possible results. If you have breast cancer and the mammogram is positive, the result is a 'true positive'. However, if you have breast cancer but the mammogram is negative, the result is a 'false negative'. If you do not have breast cancer, and the test is negative, the result is a 'true negative'. Finally, if you do not have breast cancer, but the test is positive, the result is a 'false positive'. We can arrange these possible outcomes in a 2x2 table as shown in **Figure 10.13**.

	Test Positive	Test Negative	
Disease Present	TP	FN	Number with Disease = TP+FN
Disease Absent	FP	TN	Number without Disease = FP+TN
	Number who Test Positive = TP+FP	Number who Test Negative = FN+TN	Total Number Tested = TP+FN+FP+TN

Figure 10.14: Possible outcomes of a diagnostic or screening test.

We define the sensitivity of a test as the probability that given DISEASE, the patient tests POSITIVE. The sensitivity is a measure of the ability of the test to correctly detect disease when it is present, or the ability to find true positives. Sensitivity can range from a low of 0% to a high of 100%. We define the specificity of a test as the probability that given NO DISEASE, the patient tests NEGATIVE. Specificity characterizes the ability of a test to avoid calling normal things disease, or the ability to avoid false positives. Specificity can also range from a low of 0% to a high of 100%. A perfect test has a sensitivity of 100% and a specificity of 100%. If a test performs better than chance alone (or better than the toss of a coin), the sum of the sensitivity and specificity is greater than 100%.

Table 10.10 lists the average reported sensitivity and specificity of two different screening tests for breast cancer – clinical breast exam, and mammography. The average sensitivity of mammography is 75%. This is higher than the 54% sensitivity of clinical breast exam. The specificity of mammography is 92%, slightly lower than that of clinical breast exam.[25] How do we measure the sensitivity and specificity of a screening test? If we screen a population of patients, some of whom are known to have disease and some who are known to be disease free, we can calculate the sensitivity and specificity of the test. **Figure 10.14** shows the possible outcomes of the testing.

The sensitivity can be calculated as:

	Test Positive	Test Negative	
Disease Present	TP= 11	FN = 2	Number with Disease = TP+FN = 13
Disease Absent	FP = 15	TN = 208	Number without Disease = FP+TN = 223
	Number who Test Positive = TP+FP = 26	Number who Test Negative = FN+TN= 210	Total Number Tested = TP+FN+FP+TN = 236

Figure 10.15: Data to calculate the sensitivity and specific of MRI screening for breast cancer. Used with permission from [26].

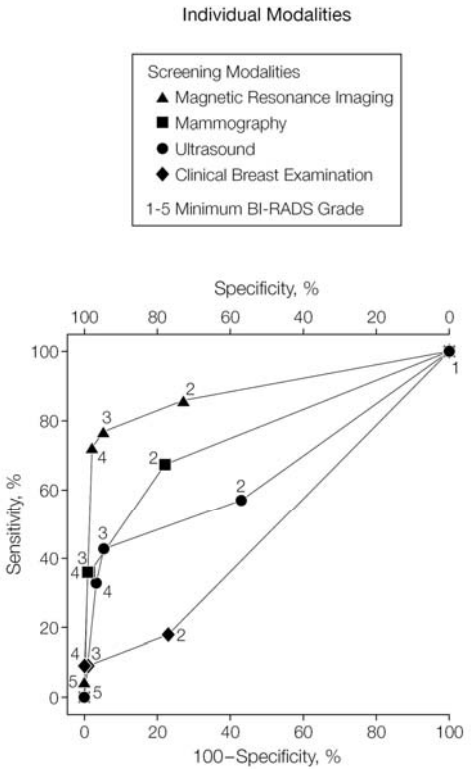


Figure 10.16: ROC curves for different breast cancer screening modalities. The area under the curve is highest for MRI. Used with permission from [26].

$$Se = \frac{TP}{(\text{number with disease})} = \frac{TP}{(TP + FN)}$$

Equation 10.1

The specificity can be calculated as:

$$Sp = \frac{TN}{(\text{number without disease})} = \frac{TN}{(TN + FP)}$$

Equation 10.2

As an example, let's calculate the sensitivity and specificity of a new test suggested for breast cancer screening—magnetic resonance imaging or MRI. In 2004, results from a clinical trial of 236 women were reported to assess the performance of MRI for screening for breast cancer in women at high risk of developing the disease.[26] During the first year of the study, each woman had an MRI exam; 26 women had an abnormal MRI. To confirm the presence of breast cancer, additional testing was performed in women with an abnormal breast MRI; these tests confirmed the presence of breast cancer in 11 women. An additional two women who had a normal MRI exam were found to have breast cancer based on other tests. **Figure 10.15** shows the 2 x 2 table filled out for this example. We can calculate the sensitivity and specificity of MRI in this clinical trial as:

$$Se = \frac{TP}{(TP + FN)} = \frac{(11)}{(11 + 2)} = 84.6\%$$

Equation 10.3

$$Sp = \frac{TN}{(TN + FP)} = \frac{(208)}{(208 + 15)} = 93.3\%$$

Table 10.11: MRI is significantly more expensive than other clinical methods of breast cancer screening. [25]

Screening Method	Medicare Reimbursement
Clinical Breast Exam	$39
Mammography	$90
Ultrasound	$74
MRI	$1108

Equation 10.4

In order to calculate the sensitivity and specificity of a new test, we must develop criteria to determine whether the test result is normal or abnormal. As these criteria change, our estimate of the test's sensitivity and specificity change. We can characterize the performance of a test by plotting the test sensitivity and specificity as we vary these criteria. The resulting plot of sensitivity vs. specificity is known as a receiver-operator characteristic curve (ROC curve). **Figure 10.16** shows the ROC curve for MRI used to screen for breast cancer in high risk women calculated from the study above.[26] As the sensitivity increases, the specificity decreases. The area under the ROC curve is often used to provide a measure of test accuracy. A perfect test has an ROC curve with area 1; a test that performs no better than chance has an area under the curve of 0.5. **Figure 10.16** also compares the ROC curves for several screening methodologies in the same group of patients; the area under the ROC curve is highest for MRI. Unfortunately, the cost of MRI is substantially higher than the cost of clinically accepted technologies (**Table 10.11**).[25] In Chapter 11, we will examine how to decide whether the additional resources required to implement a new technology represent a good investment.

As a patient, you wish to be screened with a test that has both a high sensitivity and specificity. But how high do these values need to be for the test to be useful to you? If you receive a positive screening test result, what is the likelihood that the result is a true positive or a false positive? Similarly, if you receive a negative screening test result what is the likelihood that the result is a true negative or a false negative? The sensitivity and specificity of the test don't provide enough information to answer these questions. Instead, we must calculate the positive and negative predictive value of the test, which give these probabilities.

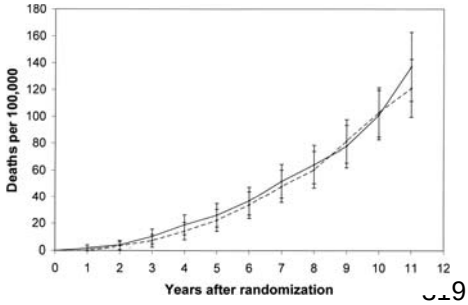
The positive predictive value (PPV) is the probability that,

Breast Cancer Screening in China: Is Breast Self-Examination an Alternative to Mammography?

While screening mammography can reduce the mortality associated with breast cancer, it is not available in many developing countries due to limited resources. In such settings, breast self-examination may provide a less expensive alternative. A recent randomized clinical trial of breast self-examination was conducted in Shanghai, China to determine whether breast self-examination could reduce breast cancer mortality.

Beginning in 1989, more than 266,000 women in Shanghai were randomized into two groups. One group of 132,979 women received initial instruction in breast self examination, with reinforcement sessions one and three years later. These women practiced breast self examination every 6 months for five years. 133,085 women were assigned to a control group. All women were followed through December 2000 for mortality from breast cancer.

The graph below shows the cumulative breast cancer mortality per 100,00 women in the breast self-examination group (solid line) and the control group (dashed line). There were a total of 135 breast cancer deaths in the group who received instruction in breast self examination, compared to 131 breast cancer deaths in the control group. In addition, more benign breast lesions were discovered in the group of women who performed breast self-examination. Unfortunately based on this study, it does not appear that breast self examination can reduce mortality from breast cancer in this setting.



given a POSITIVE test result, you have DISEASE. PPV ranges from 0-100%. The negative predictive value (NPV) is the probability that given a NEGATIVE test result, you do NOT HAVE DISEASE. Again, NPV ranges from 0-100%. We can use our 2x2 table to calculate PPV and NPV:

$$\text{PPV} = \frac{\text{TP}}{(\#\ \text{testing positive})} = \frac{\text{TP}}{(\text{TP} + \text{FP})}$$

Equation 10.5

$$\text{NPV} = \frac{\text{TN}}{(\#\ \text{testing negative})} = \frac{\text{TN}}{(\text{FN} + \text{TN})}$$

Equation 10.6

Again, we can use our MRI example of Table 11 to illustrate how to calculate positive and negative predictive value.

$$\text{PPV} = \frac{(11)}{(26)} = 42\%$$

Equation 10.7

$$\text{NPV} = \frac{(208)}{(210)} = 99\%$$

Equation 10.8

These statistics tell a patient that, given an abnormal screening MRI, there is only a 42% chance that she actually has breast cancer. Further testing is required to confirm whether cancer is actually present. However, given a normal screening MRI, there is a 99% chance that the patient truly does not have breast cancer.

Clearly, the NPV and the PPV of a test depend on the sensitivity and specificity of the test. But they also depend on the prevalence of the disease that we are screening for. Prevalence is a measure of whether a disease is common or rare. Recall that prevalence of disease in a population, p, is defined as:

Equation 10.9

In terms of our 2 x 2 table, prevalence can be calculated as: $p = \frac{(\#\text{ in population with disease})}{(\text{total }\#\text{ in population})}$

$$p = \frac{(TP + FN)}{(TP + FP + TN + FN)}$$

Equation 10.10

If we know the prevalence of a disease and the sensitivity and specificity of a test, we can calculate the positive and negative predictive values of the test as follows:

$$PPV = \frac{(p)(Se)}{[(p)(Se) + (1-p)(1-Sp)]}$$

Equation 10.11

$$NPV = \frac{(1-p)(Sp)}{[(1-p)(Sp) + (p)(1-Se)]}$$

Equation 10.12

Using our example of MRI to screen for breast cancer again, the prevalence of disease is:

$$p = \frac{(13)}{(236)} = 0.055 \text{ or } 5.5\%$$

Equation 10.13

We can use the formulas above to calculate positive and negative predictive value:

$$PPV = \frac{(0.055)(0.846)}{[(0.055)(0.846) + (0.945)(0.067)]} = 42\%$$

Equation 10.14

$$NPV = \frac{(0.945)(0.933)}{[(0.945)(0.933) + (0.055)(0.154)]} = 99\%$$

Equation 10.15

We obtain exactly the same results as calculated previously. As the prevalence of disease decreases, the PPV of a test decreases. In our example of MRI to screen for breast cancer, the study was designed to screen women who were at

high risk for breast cancer. As a result, 5.5% of the population studied had breast cancer, a prevalence that is much higher than that in the general population. Let's consider what would happen to the predictive value of the test if we were to use the same test to screen for breast cancer in all women. In the United States approximately 131 new cases of breast cancer are identified per 100,000 women.[28] Let's calculate the prevalence, positive and negative predictive value under these conditions.

The prevalence is:

$$p = \frac{(131)}{(100{,}000)} = 0.0013 = 0.13\%$$

Equation 10.16

The sensitivity and specificity are independent of disease prevalence, and as before are:

$$Se = 84.6\%$$

Equation 10.17

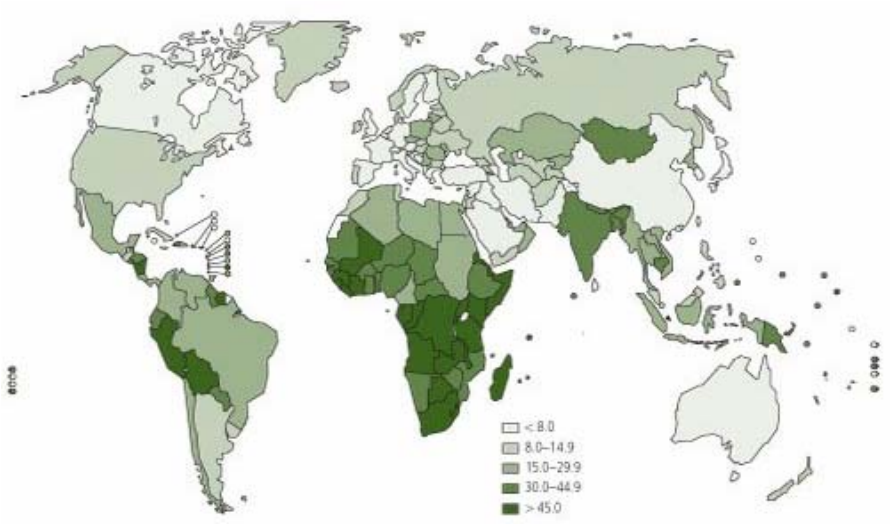
$$Sp = 93.3\%$$

Equation 10.18

However, the positive and negative predictive values differ:

Figure 10.17: Global predictions for cervical cancer mortality in 2005.

http://www.who.int/bulletin/volumes/84/2/news_fig_0206/en/index.html



$$PPV = \frac{(0.0013)(0\ .846)}{[(0.0013)(\ 0.846) + (0.99987)(\ 0.67)]} = 1.6\%$$

Equation 10.19

$$NPV = \frac{(0.99987)(\ 0.933)}{[(0.99987)\ (0.933) + (0.0013)(0\ .154)]} = 99.98\%$$

Equation 10.20

The PPV deceases substantially, while the NPV increases slightly. Suppose a woman in our study has a positive MRI. What is the likelihood that she has breast cancer? This is the same as the positive predictive value and is only 1.6%! The low PPV illustrates the challenge of screening for a rare disease. For this reason, we generally screen for breast cancer in older women, because the prevalence of breast cancer increases with age.

While screening for breast cancer using mammography clearly reduces breast cancer mortality, it has been estimated that we must screen 1224 women for more than 14 years in order to prevent one death from beast cancer. Among women between the ages of 40-49 years, we must screen 1792 women for 14 years to prevent one death from breast cancer.[29]

With this introduction, we now examine three cancers in detail – cervical cancer, prostate cancer, and ovarian cancer. In each case, we will examine the efficacy of existing screening technologies. We will also examine the new technologies in development to improve early detection.

Early Detection of Cervical Cancer:
In 2007, there are predicted to be 11,150 new cases of cervical cancer in the US, and 3,670 deaths due to cervical cancer.[1]

Worldwide, cervical cancer is an important problem. 493,000 new cases of cervical cancer were reported globally in 2002. 83% of cervical cancers occur in the developing world, with the highest incidence in central and South America, southern Africa and Asia (**Figure 10.17**). Cervical cancer caused 274,000 deaths in 2002 worldwide, and was the **leading cause of female cancer mortality in developing countries**.[6] Cervical cancer affects relatively young women, and is the single largest cause of years lost

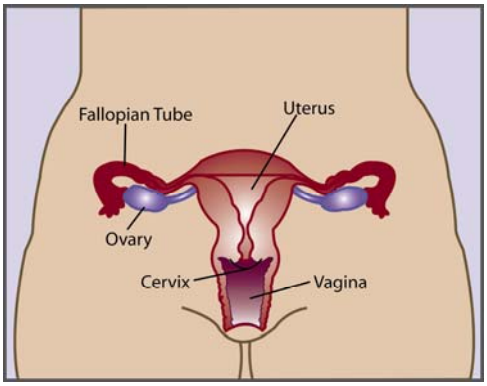


Figure 10.18: Anatomy of the female reproductive tract.

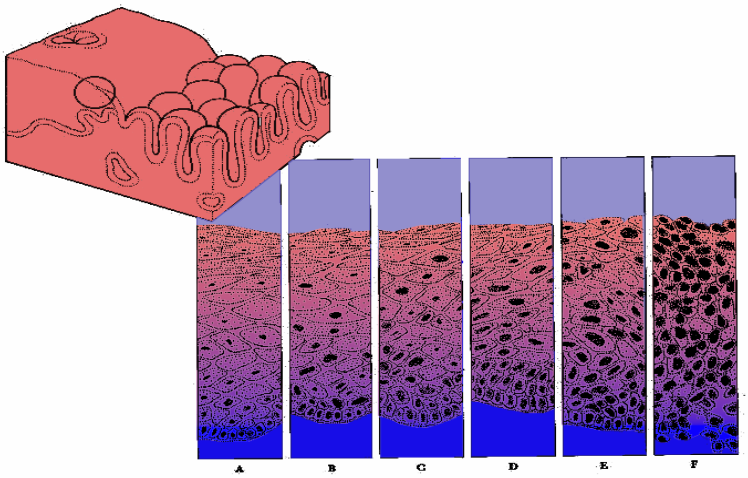


Figure 10.19: (left) a cross section showing the two types of tissues lining the cervix; (right) in normal cervical tissue squamous cells change in appearance from top to bottom (A), but in precancer this gradient becomes increasingly absent.

Figure 10.20: Fraction of cervical cancer accounted for by different types of HPV. The two vaccines under development and testing protect against HPV types 16 and 18, which together account for about 70% of cervical cancers [34].

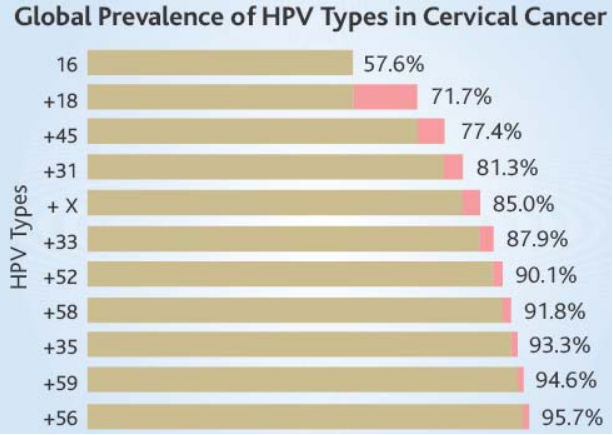
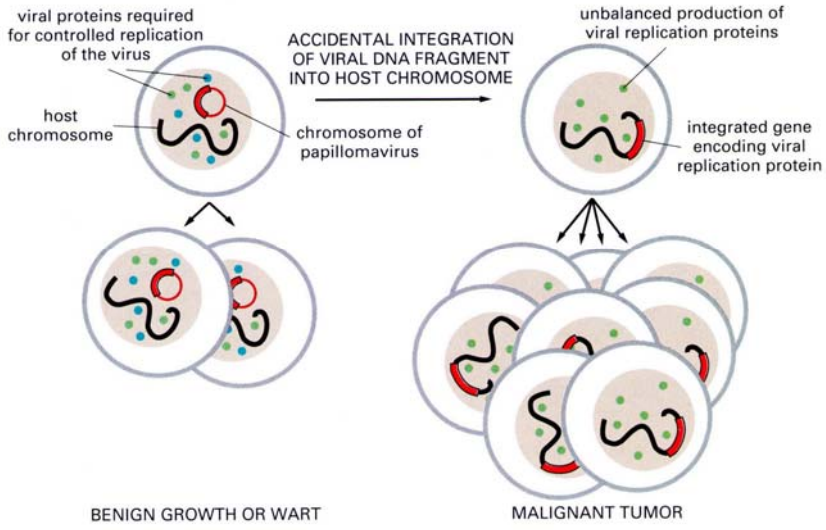


Figure 10.21: In a wart or benign infection, the HPV chromosomes are stably maintained in the basal epithelium as plasmids (left). Integration of viral DNA into a host chromosome alters the environment of the viral genes and disrupts control of their expression. Unregulated reproduction of viral proteins tends to drive the host cell into S phase helping to generate a cancer (right). Adapted from [15].

to life due to cancer in the developing world.[30]

The cervix is located between the vagina and the uterus (**Figure 10.18**). The cervical os is the opening into the uterus; during conception, sperm travel from the vagina through the os to fertilize an egg. Throughout a pregnancy, the cervix provides the structural stability to hold the fetus inside the womb. During labor and delivery, the cervix thins and stretches to enable the baby to travel through the birth canal. Thus, the wall of the cervix contains both collagen and elastin fibers to provide both strength and elasticity. The outer surface of the cervix comes into contact with both semen and potentially dangerous bacteria and viruses. The cervix is lined with multiple layers of epithelial cells that play an important role both in preventing infection and facilitating conception. These epithelial cells produce cervical mucus; the mucus changes consistency throughout the menstrual cycle in order to facilitate travel of sperm during ovulation and to prevent travel and growth of pathogens.

Cervical cancers begin in the epithelial lining of the cervix. Two types of epithelial tissue line the cervix (**Figure 10.19**); in both cases the epithelial cells are separated from the supporting stromal tissue below by a thin basement membrane. Surrounding the os, the surface of the cervix has small fingerlike projections and is lined by a single layer of columnar epithelial cells. The outer edges of the cervix are flat and lined by multiple layers of squamous epithelial cells. The squamous epithelium is typically 200-300 microns thick. The junction between the columnar and squamous epithelium is known as the squamo-columnar junction (SQ junction). Most cervical cancers begin when an epithelial cell in the squamous epithelium becomes transformed and begins to proliferate. When an epithelial cell near the basement membrane becomes transformed, it loses the capacity to terminally differentiate, and the epithelium gradually fills with actively dividing cells that have large nuclei. When the bottom 1/3 of the epithelium is filled with transformed cells, the condition is referred to as a low grade squamous intraepithelial lesion (LGSIL). When the bottom 2/3 of the epithelium is filled with transformed cells, the condition is referred to as a high grade squamous intraepithelial lesion (HGSIL), and when the complete epithelium



is transformed, it is called a carcinoma in situ (CIS). At this stage, the lesions are considered to be precancerous. However, if the transformed cells break through the basement membrane and migrate into the stroma, the condition is known as a micro-invasive cancer. This cycle is known as the precancer to cancer sequence. The prognosis of micro-invasive cancer is much more serious and the treatment is much more invasive than that of precancers. Thus, the focus of cervical cancer screening programs is to identify cervical precancers (when they can be easily treated) before they become cervical cancers (when they are difficult, painful and expensive to treat). Most low grade precancers regress on their own, while 20-45% of high grade lesions progress to cervical cancer if untreated. The progression from precancer to cancer has been estimated to take about 10-15 years.[31]

What causes transformation of cervical epithelial cells?

In the 1990s, researchers demonstrated that infection with human papillomavirus (HPV) is the central causative factor in squamous cell carcinoma of the cervix. HPV infection is the most common sexually transmitted disease; asymptomatic HPV infections can be detected in 5-40% of women of reproductive age.[32] The majority of women with HPV infection do not develop invasive cervical cancer. In most young women, HPV infections are transient; the immune system clears them with no ill effects. However, if HPV infection persists past age 30, there is a much greater risk of developing cervical cancer.[33]

There are more than 100 different types of the human papillomavirus; not all of them are carcinogenic. Fifteen types of HPV are commonly linked to cervical cancer, with HPV types 16 and 18 the most commonly found high risk types of virus (**Figure 10.20**).[34] Human papillomaviruses have double stranded circular DNA chromosomes with about 8,000 nucleotide pairs.[35] In an HPV infection, the HPV genetic material is transported to the nucleus of infected cervical epithelial cells. In a wart or benign infection, the HPV chromosomes are stably maintained in the basal epithelium as plasmids whose replication keeps step with the chromosomes of the host (**Figure 10.21, left**). A cell becomes transformed when the viral DNA is integrated into a host chromosome. This alteration of the viral gene environment can disrupt control of their expression. The unregulated production of viral proteins tends to increase the rate of cell division, thereby helping to generate a cancer (**Figure 10.21,**

US Regulations to Ensure Quality in Cytology Labs:

In the mid-1980s, a number of cases were brought to light in which women had developed cervical cancer despite having routine Pap smears with normal results. Media coverage of the cases implied that the false negatives resulted from laboratory errors due to carelessness. At the time, many commercial clinical laboratories set very high target rates for cytotechnologists to screen a certain number of slides per day or risk being fined part of their salary. Several such cases received extensive media coverage. As a result, the US Congress passed the Clinical Laboratory and Improvement Amendments of 1988 (CLIA). CLIA limited the number of slides that cytotechnologists in the US can review to no more than 100 slides per day. CLIA also mandated that 10% of slides with a "normal" diagnosis by re-screened in order to limit the number of false negative diagnoses. Sources: [37-39].

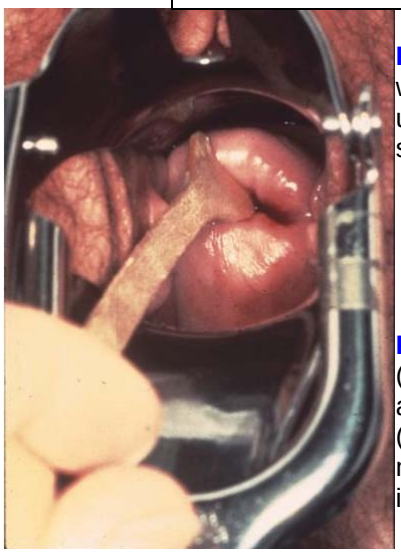


Figure 10.22: A wooden spatula is used to obtain a Pap smear sample.

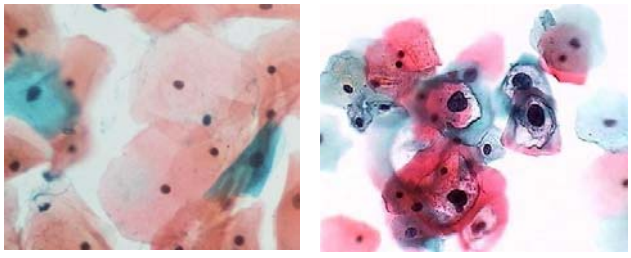


Figure 10.23: (bottom left): Normal appearing cells and (bottom right) abnormal appearing cells in a Pap smear.

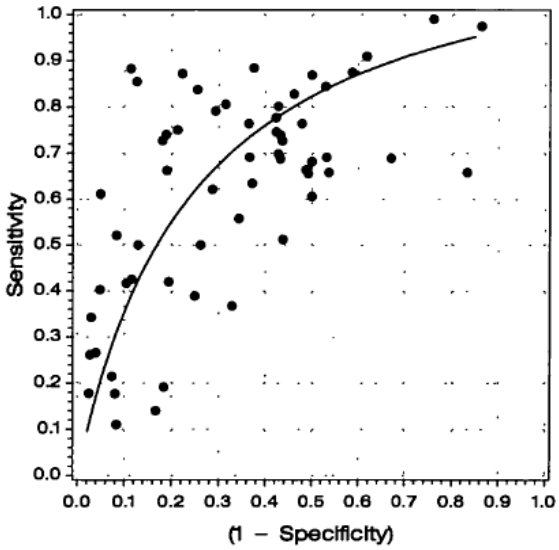
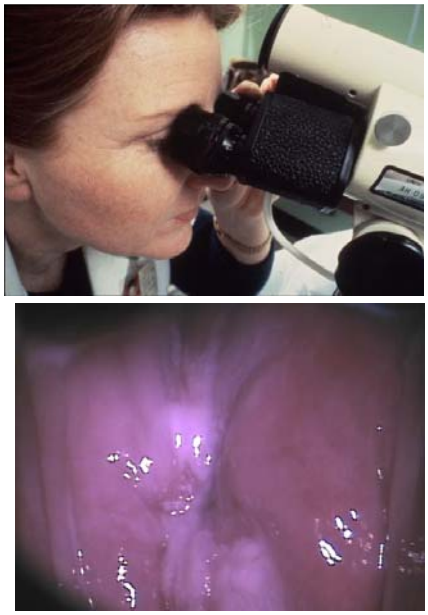


Figure 10.24: The sensitivity and specificity of the Pap smear from 62 different studies can be used to estimate the ROC curve of the Pap test. Used with permission from [41].

Figure 10.25a,b: (Top): The use of a colposcope to view the uterine cervix. (Bottom): Colposcopic photo of cervix.



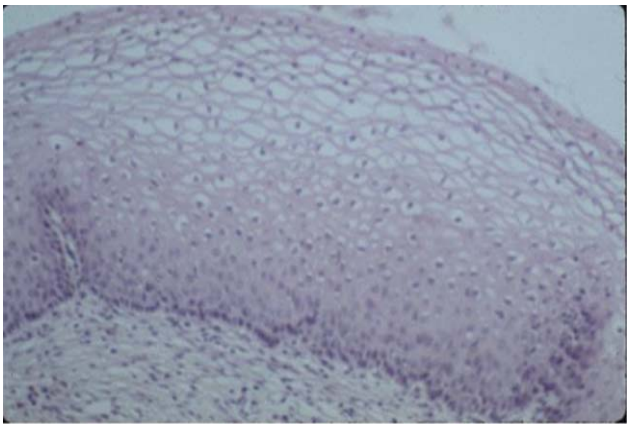
right). For example, the HPV E6 protein appears to alter cell growth through effects on p53, an endogenous tumor-suppressor protein. E6 binds to p53, targeting it for destruction. [35]

The signs and symptoms of cervical cancer include abnormal vaginal bleeding, in between periods or especially related to intercourse, and pelvic pain. Advanced cervical cancer is treated using surgery, and a combination of radiation therapy and chemotherapy. In the US, the 5 year survival rate for localized cervical cancer is excellent at 92%. Slightly more than half of cervical cancers in the US are diagnosed at this stage.[1] Such a large fraction of cervical cancers are detected early because we have a good screening test. In fact, most lesions are caught at the stage where they are still precancers, and can be treated easily before they progress to cancer.

How do we detect cervical cancer and its precursors? We screen for cervical cancer and its precursors using a test called the Papanicouloau (Pap) smear. The use of the Pap smear to screen has resulted in dramatic decreases in the incidence and mortality of cervical cancer, and is largely viewed as the most successful cancer screening tests in medical history. The diagnosis of cervical cancer and its precursors is made using a confirmatory follow-up test, colposcopy and biopsy.

In a Papanicoloau smear, a speculum is inserted into the vagina enabling the health care provider to visualize the cervix. A small wooden spatula is scraped against the cervix; the spatula is placed at the squamo-columnar junction and rotated to scrape off epithelial cells all around the junction (**Figure 10.22**). The cells collected on the spatula are then smeared on to a glass slide and allowed to dry. In obtaining a successful Pap smear, more than 50,000-300,000 cells, including both columnar and squamous epithelial cells, will be placed on the slide. The cells are then stained and examined by a trained cytotechnologist. Any abnormal appearing cells (cells with large nuclei or abnormal chromatin) are noted (**Figure 10.23**). Based on these changes, Pap smears are classified into several categories: normal, infection/repair, atypical cells of uncertain significance, low grade precancer, high grade precancer, and cancer. Interpretation of the Pap smear is subjective, and the reproducibility of this interpretation has been found to be poor. An individual clinician agrees with their own prior diagnosis about 78% of the time and agrees with the diagnosis of others only between 28-72% of the time.[36]

While Pap smears are helpful in identifying cervical cancer and its precursors, a number of factors can lead to false positive and false negative results. Only a small fraction of Pap smears



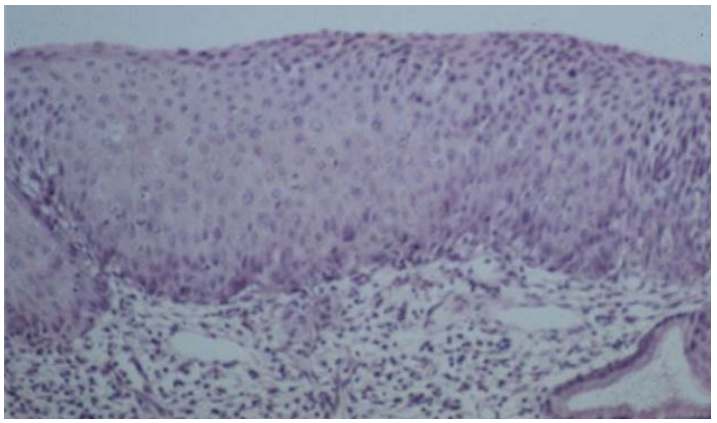
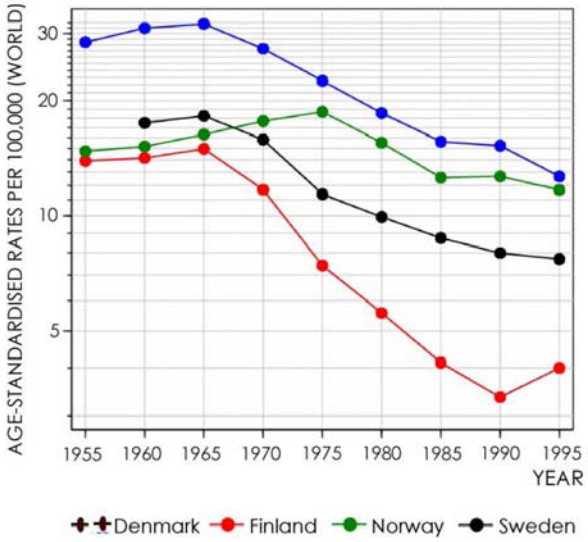


Figure 10.26a,b: (Left) photograph of a normal cervical biopsy [40]; (Right) photograph of high grade precancer [43].

contain abnormalities, and in those cases only a small percentage of cells may show cancerous changes, so positive cases are sometimes missed due to human error. Since the Pap smear samples only a fraction of cells from the cervix, abnormal cells present on the cervix may not be exfoliated when the sample is collected. Finally, benign changes such as infection or inflammation and tissue repair can cause cells to have the appearance of precancerous cells.

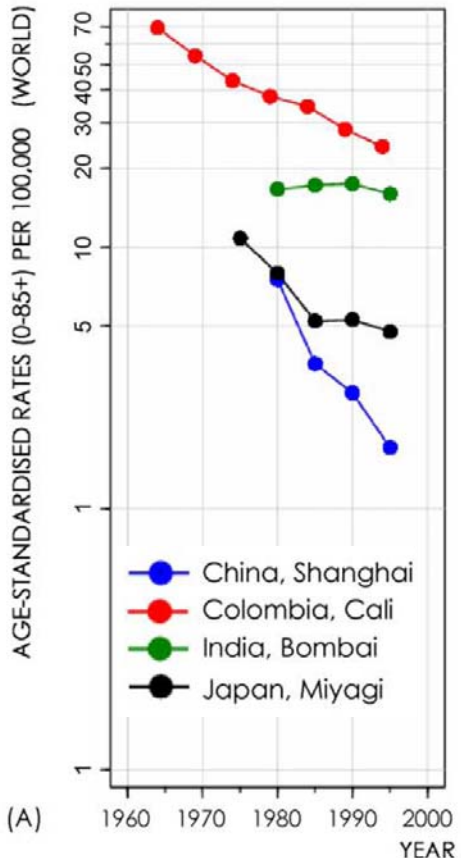
It is difficult to measure the accuracy of the Pap smear for several reasons. For example, in many studies of Pap test accuracy, only patients with an abnormal Pap smear receive further testing to confirm the presence of disease. Studies of this type suffer from what is known as verification bias; only enough data are collected to allow one to calculate the sensitivity of the test, but not the specificity. Recent studies designed to verify both positive and negative results indicate that the sensitivity of the Pap test ranges from 30% - 87% (average 47%), while the specificity ranges from 86% - 100% (average 95%) when low grade Pap smears and worse are considered to be abnormal. [40] **Figure 10.24** shows sensitivity and specificity of 62 different studies comparing Pap test results with biopsy.[41] The sensitivity and specificity vary widely from one study to another, and it is clear that it is difficult to achieve simultaneously high sensitivity and specificity using the Pap test.

Because the Pap smear is a screening test, an abnormal Pap smear is usually followed by a diagnostic procedure called colposcopy. In colposcopy, a speculum is inserted and a low power microscope (called a colposcope) is used to view the cervix (**Figure 10.25a,b**). A solution of weak acetic acid (vinegar) is applied to the cervix. The vinegar washes away any cervical mucus and also causes any precancerous areas of tissue to turn white. Any suspicious areas on the cervix are then biopsied, using a metal biopsy forceps to remove a pea-sized portion of tissue. The biopsy is cut, stained and examined under the microscope by a pathologist. The sensitivity of visual



The article was published in Vaccine, Vol. 24S3, D. Maxwell Parkin and Freddie Bray, The burden of HPV-related cancers, pg. S3/11-S3/25, © Elsevier (2006).

Figure 10.27: Incidence rates of cervical cancer in four Nordic countries. Decreases in the incidence rate parallel the introduction and extent of screening programs.



The article was published in Vaccine, Vol. 24S3, D. Maxwell Parkin and Freddie Bray, The burden of HPV-related cancers, pg. S3/11-S3/25, © Elsevier (2006).

Figure 10.28: Incidence rates of cervical cancer in four countries. Where screening programs are not available, incidence rates have been stable.

examination using the colposcope is excellent, at 96%; however, the specificity is quite low, only 48%.[42] The low specificity of colposcopy is the reason that a confirmatory biopsy must be obtained; but due to the low specificity, more than half of all biopsies obtained at colposcopy show only benign changes.

Figure 10.26a shows a histologic section of normal cervix from the squamous epithelium prepared from a biopsy obtained under colposcopic guidance; the cervix is lined by about 10-15 layers of epithelial cells. In the normal cervix, the basal layer of cells has the largest N/C ratio. The cells at the top of the epithelium have the smallest N/C ratio. **Figure 10.26b** shows a histologic section of a high grade precancer. Clearly, the N/C ratio is increased throughout the entire epithelium. In this specimen, the cells have not yet invaded the basement membrane to form a micro-invasive cancer.

In summary, we screen for cervical cancer and its precursors using the Pap smear, and we confirm the diagnosis using colposcopy and biopsy. Precancerous cervical lesions can be removed using a simple outpatient electro-surgical procedure to remove the transformed epithelium; this treatment preserves fertility. Because we have a good screening test, most lesions are caught at the stage where they are still precancers, and can be treated easily before they progress to cancer. As a result, by screening for precancer, we can actually reduce the incidence of cervical cancer.

Before the introduction of screening programs, the incidence of cervical cancer in North America and Europe was similar to that seen in developing countries today. In every country in which organized screening programs based on the Pap smear have been introduced, rates of cervical cancer incidence and mortality have decreased.[30] While cervical cancer screening has never been tested in randomized clinical trials, reductions in cervical cancer mortality and incidence in countries where screening is practiced provide evidence that screening is effective. **Figure 10.27** shows the declines in cervical cancer incidence in Nordic countries where screening programs were introduced in the 1960s to 1970s. **Figure 10.28** compares changes in incidence rates over time in four countries. Decreases in Shanghai, China reflect the introduction of an intensive screening program; in contrast, incidence rates have been stable in Bombay, India where screening is largely unavailable. [30]

The Pap smear is viewed as one of the most successful public health measures ever introduced. Given the relatively low sensitivity and specificity of the Pap test, it is sometimes surprising that screening has been so successful in reducing the inci-

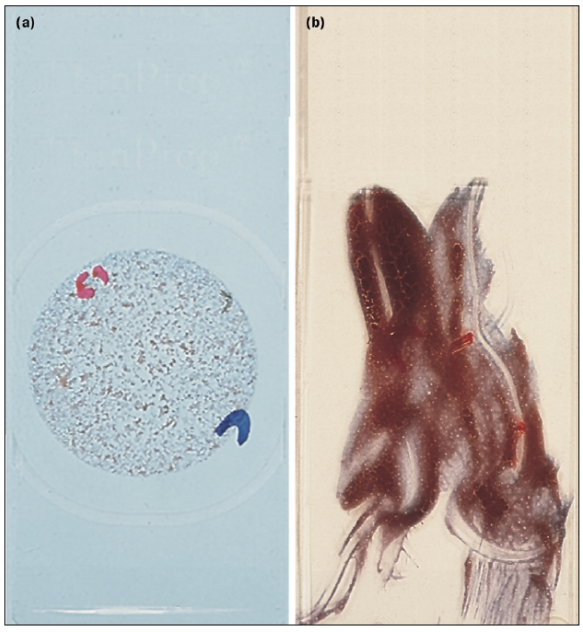
dence and mortality of cervical cancer. In large part, this is due to the fact that, on average, to go from cervical precancer to invasive cervical cancer requires 8 years.[44] Even if a woman has a falsely negative Pap smear one year, chances are it will be detected when she has her next Pap smear, before it has progressed to cancer. However, the Pap smear does miss some cervical cancers. It has been estimated that 3% of preventable cervical cancer deaths can be traced to false-negative readings; an estimated 50% of these are due to sampling error that would not be detected with re-screening. [45] We spend more than $6B annually in the US following up low grade Pap smears that likely will not yield any health benefits.[46]

Because of the costs and infrastructure requirements associated with the test, the Pap smear is not available to a large segment of the world's population and cervical cancer continues to kill many young women. There are many barriers to cervical cancer screening in developing countries; developing countries face a lack of trained cytotechnologists and cytology labs. A further complication is the lack of facilities to follow-up abnormal Pap smears and treat precancerous lesions. It is difficult for women who live in rural areas to come for multiple visits required to screen, diagnose and treat cervical cancer and its precursors. Finally, the costs of screening in many developing countries exceeds a family's daily income, putting the test out of reach for most.[47]

A number of new technologies have been developed to address the limitations of the Pap smear. In large part, these technologies have three goals: (1) reduce the false positive and false negative rates of the test, (2) develop tests that can give instantaneous results so that women could be treated at the initial visit if the test were positive, and (3) reduce the costs of the test so that it can be implemented in the developing world. Here, we will examine four new technologies to screen for cervical cancer: liquid based cytology, automated Pap smears, HPV testing, and optical testing.

Liquid Cytology: One of the primary limitations of the conventional Pap smear is that only a fraction (estimated to be 20%) of the cells collected are transferred onto the slide which is later stained and examined for transformed cells.[44] A new technique called thin-layer, liquid based cytology has been developed to improve the conventional Pap smear. In this technique, the brush used to collect the Pap smear is dipped into a vial containing a cell preservative, ensuring that all cells which are collected are available for analysis. A robotic preparation device is then used to remove blood and inflammatory cells and transfer a thin layer of representative cells in a circular area onto a slide. In one liquid cytology procedure (ThinPrep®), a

Figure 10.29: (a) ThinPrep Pap and (b) Conventional Pap smear. Used with permission from [44].



How often should women be screened using the Pap smear?

The American Cancer Society issued Screening Guidelines for the Early Detection of Cervical Cancer in 2002. These guidelines recommend that screening should begin approximately three years after women begin having vaginal intercourse, but no later than 21 years of age. Screening should be done every year with regular Pap tests or every two years using liquid-based cytology. If a woman has had three normal tests in a row and has reached 30 years of age, she may reduce the frequency of screening to every 2-3 years. However, doctors may suggest a woman get screened more often if she has certain risk factors, such as HIV infection or a weakened immune system. Women 70 and older who have had three or more consecutive Pap tests in the last ten years may choose to stop cervical cancer screening.

Do women follow these recommendations? 79% of women in the US report having had a Pap smear in the last 3 years. Adherence to screening is slightly lower for women with no health insurance and for women with less than a high school education.

Sources: [14,50].

Figure 10.30: An automated Pap smear machine.

spinning cylinder is lowered into the specimen vial and used to break up any clumps of cells. The cell suspension is then drawn upward through a polycarbonate filter until an approximate single layer of cells covers the filter. The filter is then briefly adhered to a glass slide in order to transfer the cells from the filter to the slide. Several slides, each containing a representative population of the exfoliated cells can be prepared from a single suspension.[48]

Figure 10.29 shows a photograph of slides prepared using the conventional manner (right) and using the ThinPrep® device (left). Trials comparing conventional Pap to a thin layer Pap showed that the thin-layer method results in an overall 18% higher detection rate of abnormalities than conventional cytology. Based on these results, this new technology was approved by the US FDA in 1996. Further studies indicate that the use of the thin-layer Pap decreases the proportion of inadequate specimens, improves the sensitivity, and reduces the specimen interpretation time compared to the conventional Pap smear.[44] There is additional cost associated with the preparation of the liquid based cytology specimen. A conventional Pap costs around $15; ThinPrep adds about $15-25 to this cost.[49]

Automated Pap Smears: Currently, Pap smears are examined by highly trained cytotechnologists. A significant amount of training is required to be able to accurately identify smears containing precancerous or cancerous cells. The lack of trained personnel is a barrier to screening in many developing countries.[47] Automated cytology devices use a microscope with autofocus and a motorized, computer-controlled stage coupled to a high resolution video camera (**Figure 10.30**). Digital images are captured and sent to a computer, where image processing algorithms are applied to interpret the images and classify the slides. Images are first segmented, to separate cells from background objects, like debris or inflammatory cells. Morphologic parameters are then calculated such as the cell size, nuclear size, the nuclear to cytoplasmic ratio and the texture of chromatin within the nucleus. In addition to features that cytologists normally use, more advanced morphologic parameters can be calculated. Abnormalities can then be detected by comparing the distributions of measured cells to those of known normal and abnormal reference cases. Classification algorithms are used to combine measured parameters and make a determination of whether the specimen is normal or abnormal. Statistically based algorithms, hierarchical decision trees or neural networks are examples of types of classifiers, each of which consists of a set of rules to classify the data.[48]

In 1998, the FDA approved the use of such a device called the

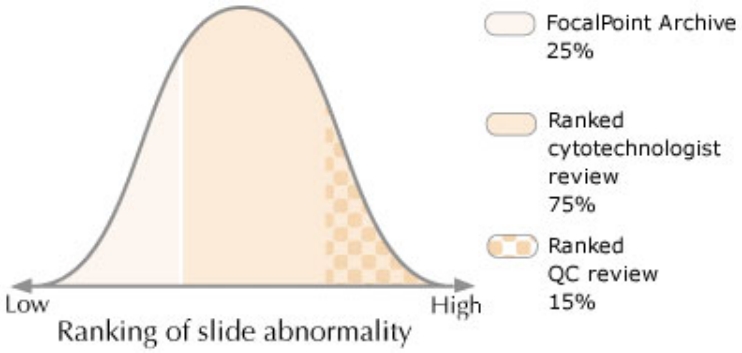


Figure 10.31: Slides obtained from a Pap smear are ranked according to abnormality before being reviewed by a cytotechnologist [44].

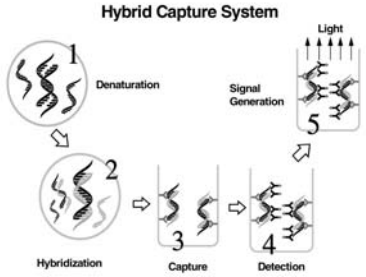
AutoPap® Primary Screening System to sort out 25% of smears that do not require human review because they are negative. The device can scan about 200 slides per day.[44] Slides containing potential abnormalities are ranked in order of abnormality. Slides with the lowest probability of abnormality are not ranked and reported as requiring "no further review." This approach can reduce the workload of the cytotechnologist, allowing him or her to focus on those slides most likely to contain abnormalities. In addition, the device is used to rank the 15% of slides with the greatest likelihood of abnormalities for re-review (**Figure 10.31**). These slides may be used instead of the 10% random selection of slides for quality control as mandated by CLIA.[51]

Clinical studies of this technology have shown that the AutoPap® device outperformed human review by a factor of 5 to 7 times when used to rescreen the 10% of negative Pap smears which must be examined for quality control purposes.[44] The use of AutoPap® adds between $3-$10 to the cost of the Pap test.[52]

***HPV DNA Testing*:** Cervical cancer is caused by infection with the human papilloma virus (HPV). A new test has been developed to determine whether a patient is infected with HPV. Following a Pap smear, the remaining material on the spatula can be tested to determine if HPV DNA is present. The DNAwithPap™ Test is FDA-approved for routine adjunctive screening with a Pap test for women age 30 and older.[53] HPV is found so frequently in women under the age of 30 that it is not useful to indicate risk of cervical cancer and its precursors. However, if viral infection persists after age 30, there is an association with increased risk of cervical cancer and its precursors. Clinical studies have shown that the sensitivity of DNAwithPap™ is greater than that of the Pap smear alone or a liquid Pap. The sensitivity of DNAwithPap™ is 80-90%, while the specificity is 57-89%.[54] In Europe, as of 2006, the use of HPV tests is not currently included in basic screening.[55]

VIA: The use of Visual Inspection with Acetic Acid (VIA), is being explored as an alternative to Pap smear and colposcopic

HPV DNA Testing: How does it work?



As of 2007, Digene's hc2 High-Risk HPV DNA Test™ (DNAwithPap™), based on Hybrid Capture® 2 technology, is the only FDA approved method for HPV DNA testing. When used for the purpose of cervical cancer screening, the FDA requires that it only be used in women over the age of 30, in conjunction with a Pap test. In most cases, HPV DNA testing may be performed on the same sample of cells collected for the Pap test. Following collection, DNA is extracted from the cell sample and denatured and single stranded RNA probes for the 13 highly-oncogenic types of HPV are added. If HPV DNA is present, it hybridizes to the probe RNA. Antibodies specific to DNA-RNA hybrids capture the hybrids and bind them to the wells of a microtiter plate. The complex is then enzymatically digested resulting in the emission of light produced by a chemiluminescent substrate conjugated to the enzyme. The intensity of light indicates the presence or absence of HPV DNA in the patient's sample.

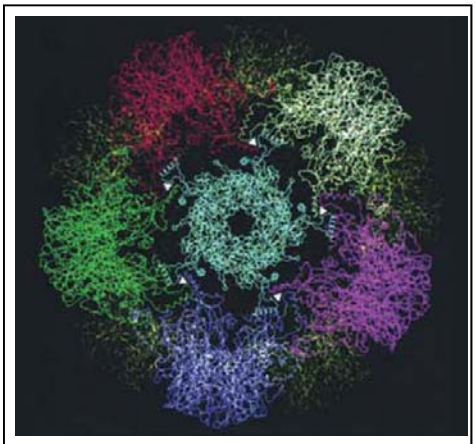
Sources: [44, 53, 56].

Vaccines to Prevent HPV Infection and Cervical Cancer:

HPV is the most common sexually transmitted disease in the US. Today, more than 20 million people in the US harbor HPV. 80% of women will test positive for HPV by age 50. As we have seen, HPV infection usually does not cause any symptoms, but in some cases it can lead to cervical cancer.

In 2006, a new vaccine to prevent HPV infection was licensed for use in girls and women aged 9 to 26 years in the US. The vaccine, Gardasil, protects against four strains of HPV. Two of these HPV types (16, 18) are responsible for 70% of cervical cancers; combined, the 4 HPV strains covered by Gardasil account for about 90% of genital warts. At the end of 2006, Gardasil had been approved in 49 countries.

Gardasil is made by inserting the gene for a protein found in the HPV capsid (L1) into a different virus or yeast. Recombinantly produced HPV capsid protein then self assembles into virus-like particles. While these empty shells do not contain the cancer causing DNA of HPV, their shape is sufficiently similar to that of the HPV virus so that the immune system triggers a protective response against future HPV infection.



VLPs made from the L1 protein of HPV 16 assemble into virus like particles. Their outer structure resembles HPV, but they do not contain HPV DNA.

http://www.cdc.gov/std/HPV/STDFact-HPV-vaccine-hcp.htm

While Gardasil can protect against new HPV infections, it is not effective for women who have already been exposed to HPV. The length of time that patients will be protected following vaccination is currently not known. As of 2006, the vaccine had been tested in more than 3,000 women who had been followed for 5 years. The vaccine was protective throughout this period, but it is not known whether booster shots will be required over longer periods of time. Trials of Gardasil and a second promising HPV vaccine made by Glaxo SmithKline (GSK) are currently underway in more than 50,000 subjects.

Currently, Gardasil is given as a series of three shots over a 6 month period; the cost of the vaccine is $360. This cost is a barrier even in developed countries, and is likely to limit its immediate impact in developing countries. For example, the HBV vaccine was licensed in 1981 in industrialized countries, but took 10-15 years for it to be used in wealthier developing countries and over 20 years before children in poor developing countries had widespread access to the vaccine. Developing countries may also face difficulties in providing widespread access to a vaccine that is targeted towards girls and young women. Vaccines for adolescents are often given through school programs, but girls in developing countries are less likely to be in school than boys. Gender specific immunization may be culturally unacceptable in some settings. Many vaccination programs have been damaged due to rumors that vaccination is a plot to sterilize girls. The stigma associated with a vaccine targeted against an STD may exacerbate such rumors. Such rumors derailed polio eradication campaigns in Nigeria and India, resulting in global consequences.

HPV Vaccine Efficacy Trials

Manufacturer	Vaccine	Location	Participants	Projected End
Merck 	VLPs of L1 protein from HPV 6/11/16/18, made in yeast, aluminum adjuvant	U.S., S. America, Europe	17,800 women, 16 to 26 years old	2007
		U.S., S. America, Europe, Asia	3800 women, 24 to 45 years old	2008
		U.S., S. America, Europe, Asia, Africa	3700 men, 16 to 24 years old	2008
GSK	VLPs of L1 protein from HPV 16/18, made in baculovirus, AS04 adjuvant	U.S., S. America, Europe, Asia Pacific	18,000 women, 15 to 25 years old	2010
		Costa Rica (run by NCI)	12,000 women, 18 to 25 years old	2010

Will the HPV vaccine eliminate the need for cervical cancer screening? Currently available vaccines do not protect against all types of HPV that cause cervical cancer, so women who receive the HPV vaccine will still need to be screened for cervical cancer. Additionally, if women don't get all three doses of the vaccine or if they have already been exposed to HPV prior to being vaccinated they may not be protected. Sources: [58-60].

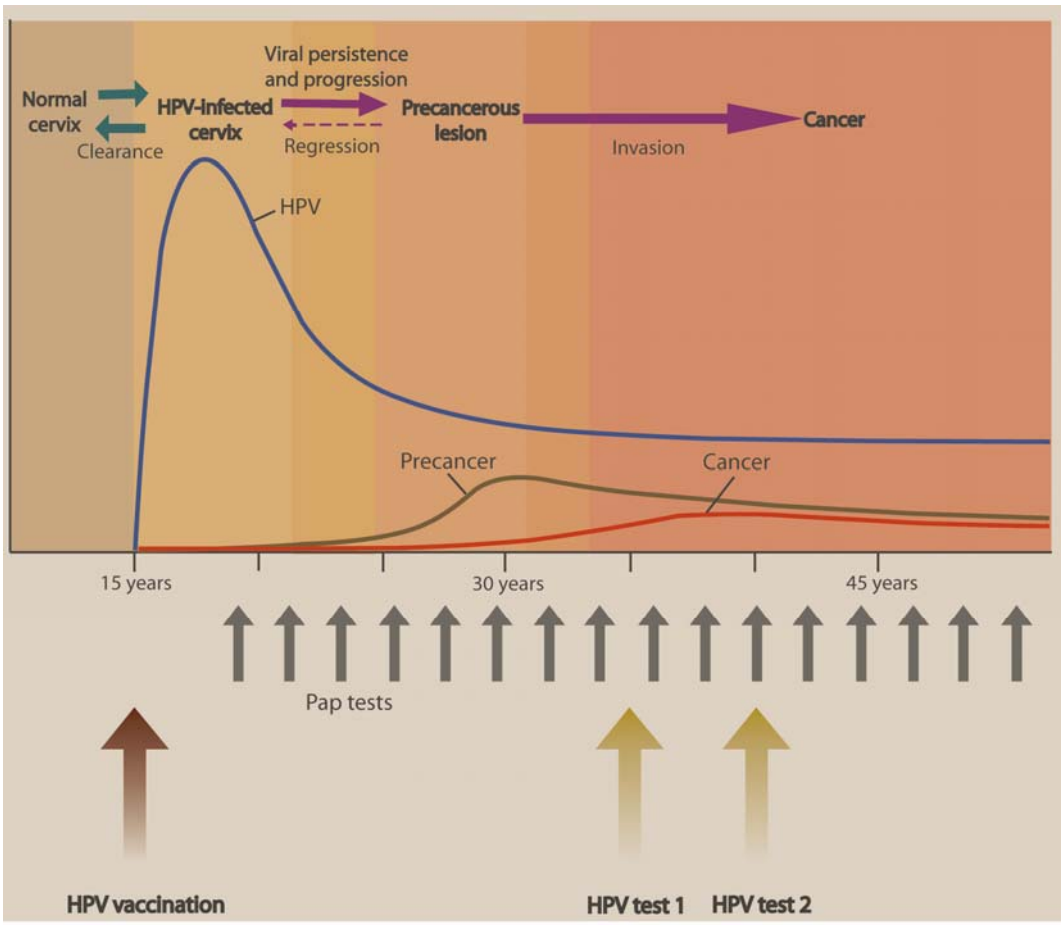


Figure 10.32: Many young women develop an HPV infection during adolescence or young adulthood. In some women, HPV infection leads to precancerous changes in the cervix. Regular Pap tests can identify these precancerous changes, allowing treatment before cervical cancer develops. In the future, the availability of an HPV vaccine may reduce the incidence of HPV infection and reduce the frequency with which screening is needed. Alternative methods of screening, such as the HPV DNA test, may improve the sensitivity of screening.

From NEJM 353:22101-2104, 2005.

ex-amination in many developing countries. VIA consists of simple visual examination of the cervix with the naked eye by a trained health care provider before and after application of acetic acid. VIA relies on the acetowhitening of precancerous lesions. It requires only low technology equipment, and results are available in a few minutes. A recent review of the performance of VIA in 9 studies involving more than 40,000 women in South Africa, India, Zimbabwe, China, and the Philippines found that VIA has similar sensitivity to that of Pap smear screening, but lower specificity, although some studies suffered from verification bias.[57]

In a study of 18,675 women in India, Sankaranarayanan found that the sensitivity of VIA for detection of high grade squamous intraepithelial lesions (HSIL) was 60.3% and the specificity was 86.8% relative to the gold standard of colposcopic directed biopsy of colposcopically abnormal lesions.[61] The advantage of VIA is that it is an inexpensive test that does not require lab infrastructure. Providers can be trained to perform the test in 5-10 days. Consumables required are cheap and universally available. Because results are available immediately, patients can be treated at the same visit. However, there are concerns

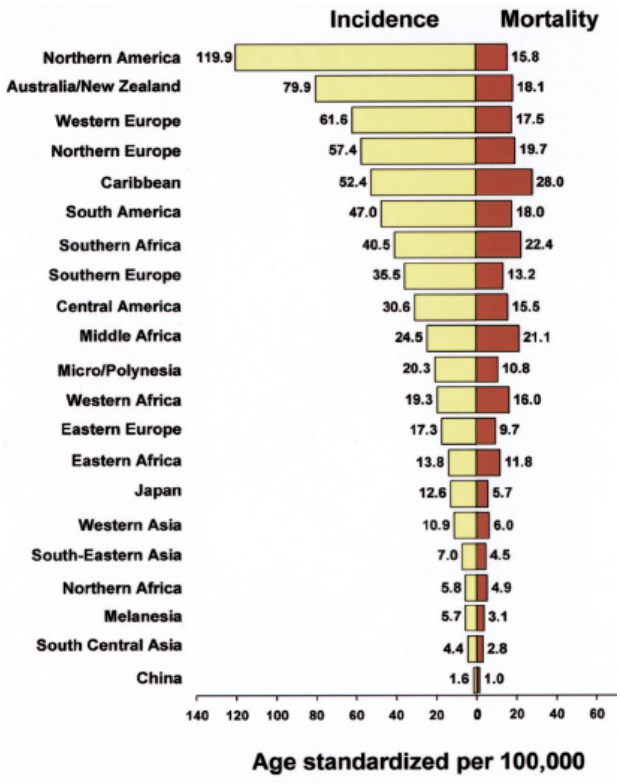


Figure 10.33: The incidence of prostate cancer is highest in North America. Mortality rates of prostate cancer are substantially lower than incidence rates.

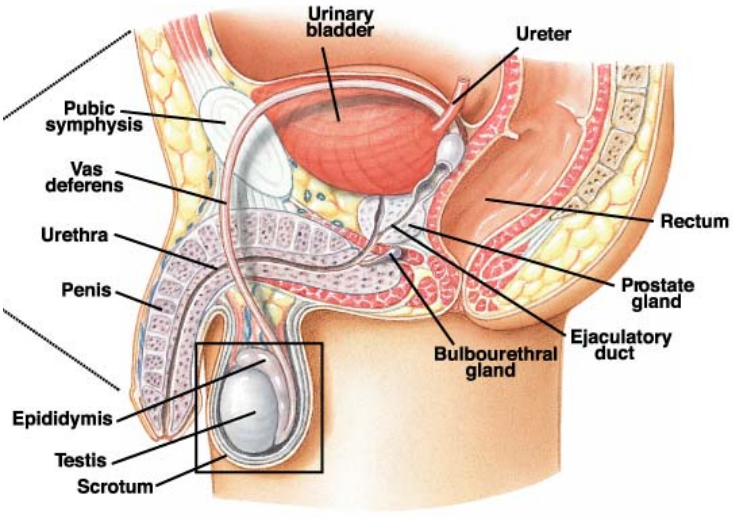


Figure 10.34: A diagram of the prostate gland in the human male.

that the low specificity of VIA may lead to over-diagnosis and treatment.[62]

Because VIA relies on visual interpretation, defining objective criteria for a positive lesion and training operators to correctly implement these criteria are crucial. In a series of 1,921 women screened in Peru, Jeronimo found that the VIA positivity rate dropped from 13.5% in the first months to 4% during subsequent months of a two year study; the drop in positivity rate was hypothesized to be due to a learning curve for the evaluator.[47]

DIA: The use of digital image analysis (DIA) may provide a simple solution to reduce the subjectivity and improve the specificity of VIA. Advances in consumer electronics have led to inexpensive, high dynamic range CCD cameras with excellent low light sensitivity. At the same time, advances in vision chip technology allow high quality image processing in real time. These advances may enable acquisition of digital images of the cervix in a relatively inexpensive way, with or without magnification. Moreover, automated image diagnosis algorithms based on modern image processing techniques has the potential to replace clinical expertise, which may reduce a considerable amount of the system cost. A recent pilot study showed that digital images of the cervix can be obtained using a simple and inexpensive device, and that automated image analysis algorithms correctly identify histologically neoplastic tissue areas with a sensitivity of 79% and a specificity of 88%. [63]

In summary, although cervical cancer is a completely preventable disease, it is the 3rd leading cause of cancer death in women in the world.[6] Cervical cancer is caused by infection with HPV. HPV infection can initiate a transformation that results in a precancerous lesion. If we detect and treat these common precancerous lesions, we can prevent the development of cervical cancer. Current screening and detection using the Pap smear followed by colposcopy and biopsy has been proven to reduce both the incidence and mortality of cervical cancer. However, we have insufficient resources to screen using these technologies in developing countries. New technologies, such as auto-

mated reading of Pap smears, HPV testing, visual inspection, and digital image analysis (VIA and DIA) technologies may provide the improvements in performance at a sufficiently low cost to enable screening in resource poor settings where the vast majority of cervical cancer occur. Coupled with vaccines to prevent HPV infection, these technologies have the potential to reduce both the incidence and mortality of cervical cancer (**Figure 10.32**).

Part II: Peace Corps and Rice Visits: July 2 - July 4, 2007

Tessa **Swaziland**

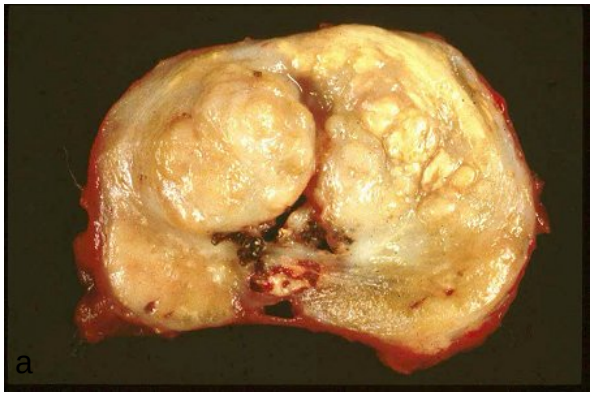
The next day, Tandi took me to the NCP (Neighborhood Care Point). There were several in the community, but this one in particular was also the kagogo, which is the central meeting place for the community. I met the secretary of the kagogo, and he asked me lots of questions about Baylor. No one in the community knew about the clinic, and he was curious as to who was eligible to go there, if it was free, and how they could become an outreach site. Most of the people wouldn't be able to afford the 42 rand (US $6) it would take to go to and from the clinic, so ideally, he would get Baylor to come to the community.

The NCPs are where orphans and vulnerable children can come for the day to receive meals and a bit of education. Tandi said that the community was pretty good about taking in the children but struggled to support them. Then NCPs filled this gap and provided as much support as possible, although often, it isn't enough either. Talia (A Canadian who visited with Rachel and Lindsay) worked a lot with orphans in Botswana. One of the services her NGO provided was gift baskets for the orphans. Once families learned about this, they started taking in as many orphans as they could in order to receive the baskets, which they would then sell. The orphans remained just as abandoned and starving as before. Tandi said that this wasn't really a problem in her community, but there were many others. For example, an orphan could go to school if they could prove (with death certificates) that they were indeed orphans. This is virtually impossible for many reasons. Many of them never knew their fathers, who left the mother when she was pregnant. Even if they knew both of their parents, no one gets a death certificate unless they go to a city far away and deal with some complicated legal procedure. So, unless there is someone who cares enough for the child to deal with the hassle and who is wealthy enough to afford it, there is no way for the orphan to prove that they lack parents. Thus, all they are left with are the NCPs.

I

listened for a bit as Tandi and the secretary discussed some of their projects—a community garden, fundraising for NCP renovations (most of them were dirty, stick-in-the-mud structures), education campaigns, and other events. During this conversation, I discovered that children become sexually active as early as twelve. I also learned that men fear the HIV stigma more than women (probably because it might limit the number of girlfriends they could have), while women were much more open and willing to address the problem. He told us that there was an article in the paper about a doctor who was telling many of his patients that they weren't actually HIV-positive even though they'd been told at a VCT (volunteer counseling and testing) clinic that they were. He thought they were lying about it because they were afraid that they would lose their jobs if the HIV rate dropped and funding for HIV/AIDS programs dropped. Tandi responded that it is much more likely that the one doctor was lying than everyone at the VCTs, and in addition, many people try to place blame elsewhere in order to avoid taking responsibility for their actions (which caused them to get the disease).

After that, I visited the school and nearby clinic. They were pretty much what I expected—about the same as the Vuvulane clinic, and the school was much like the school I worked at in Nicaragua. We looked at the picture of the map Tandi was painting with her class and I took photos of some of the HIV/AIDS awareness signs. Realizing that her watch had stopped, we rushed off to catch a combie and make our way back to Manzini. At that point, we split up. She headed over to her friend's community to help with a workshop, and I headed back to the COE in Mbabane.



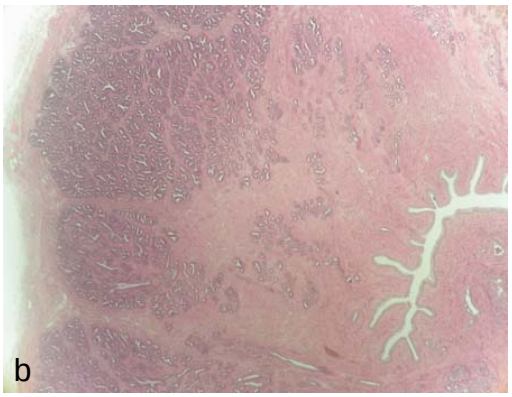
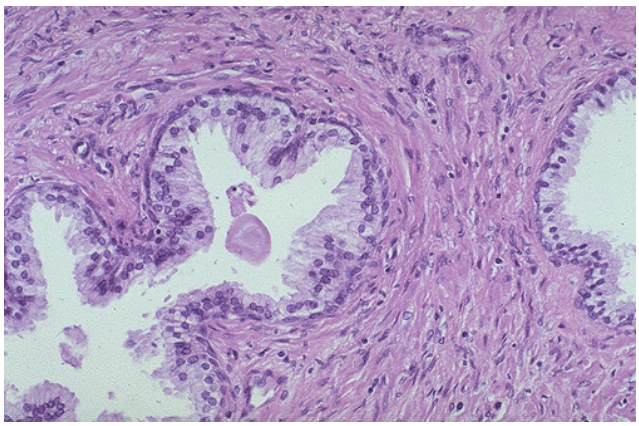


Figure 10.35: (a) shows a photograph of the normal prostate; (b) shows a histologically stained section of a normal prostate.

Prostate Cancer: As we have seen, prostate cancer is the most common cancer diagnosed in men in the US, with 218,890 new cases diagnosed annually. Prostate cancer is the 2nd leading cause of cancer death in men, causing more than 27,050 deaths each year in the United States.[1] Worldwide, more than 679,023 new cases of prostate cancer are detected annually, making prostate cancer the second most common cancer in men.[6] **Figure 10.33** shows the incidence and mortality rates of prostate cancer throughout the world. Risk factors for development of prostate cancer include advanced age, race (incidence rates are one and a half times higher in African Americans), and a family history of prostate cancer.[1]

Figure 10.34 shows the location of the prostate gland. The prostate gland contributes enzymes, nutrients and other secretions to semen. **Figure 10.35a** shows a photograph of the normal prostate, while **Figure 10.35b** shows histologically stained sections of normal prostate tissue. The normal prostate consists of several branched glands leading to the urethra. These glands are covered by a single layer of columnar epithelial cells. In the normal prostate, the nuclei of these



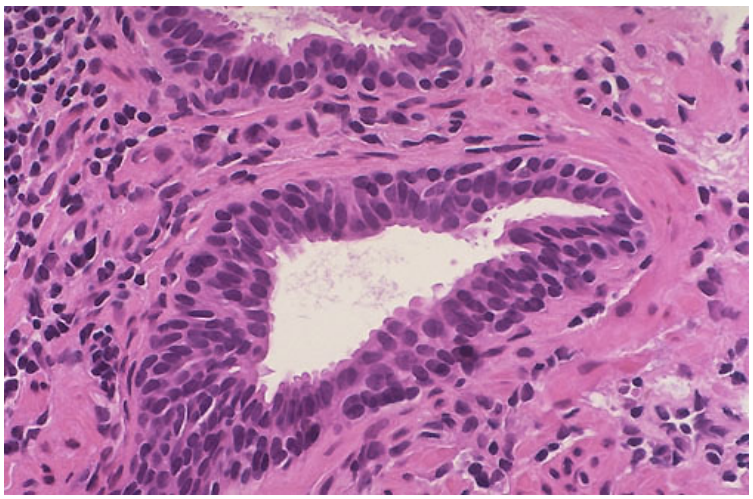
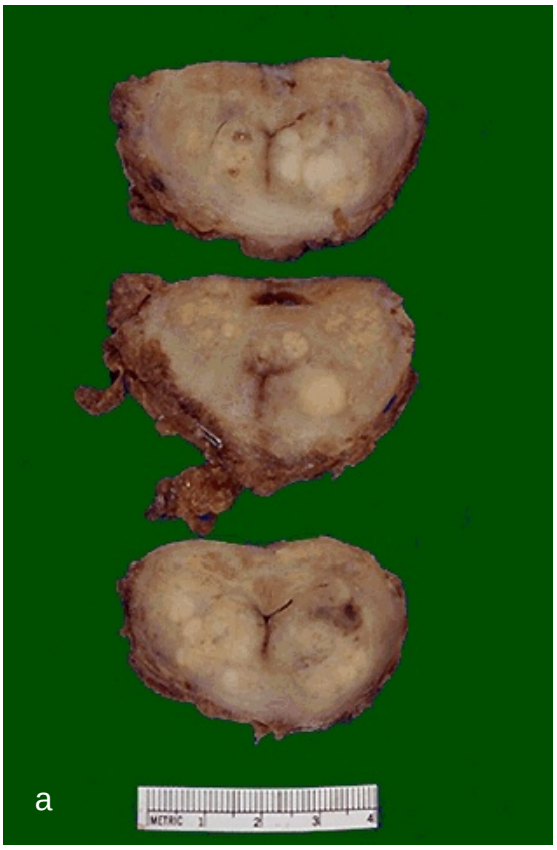


Figure 10.36: A slide showing a normal prostate; note that the nuclei of normal cells take up roughly a quarter of the cell area.

Figure 10.37: A slide showing a precancerous prostate gland, in which the nuclei of the cells have become enlarged.



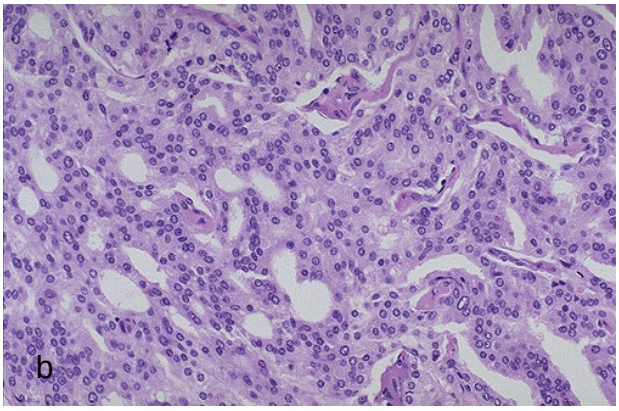


Figure 10.38: Invasive prostate cancer at the macroscopic (a) and microscopic (b) levels.

cells occupy approximately ¼ of the cell area (**Figure 10.36**). However, in precancerous lesions, the nuclei of these epithelial cells become substantially enlarged (**Figure 10.37**) and multiple layers of cells stack atop one another. As these cells invade beneath the basement membrane lining the ducts, invasive prostate cancer develops. Initially, the lesion is localized to the prostate; at the microscopic level, the cancerous epithelial cells are found throughout the entire prostate (**Figures 10.38a,b**).

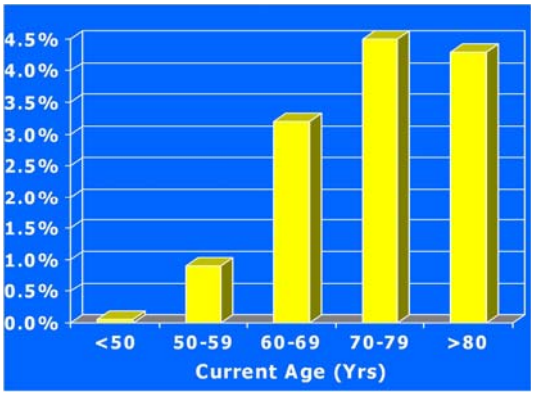


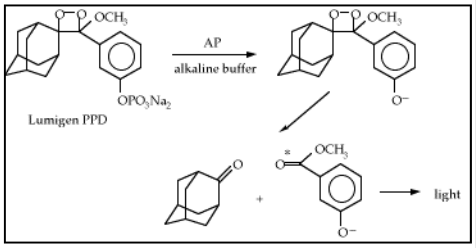
Figure 10.39: As the graph above shows, the risk of developing prostate cancer in the next 5 years increases dramatically with age.

Prostate cancer is a slow, but continuously growing cancer. Generally, preclinical asymptomatic forms of the disease can develop as early as age 30. This disease can remain latent for up to 20 years. In some patients, precancerous lesions can progress to aggressive, malignant cancer. The peak incidence of prostate cancer occurs in the 7th decade of life.[11] **Figure 10.39** shows the risk of developing prostate cancer in the next 5 years as a function of a patient's current age; the risk rises dramatically with increasing age. Prostate cancer is often asymptomatic in the early stages. When present, the signs and symptoms of prostate cancer include weak or interrupted urine flow or the inability to urinate. These symptoms are the same as those of prostate enlargement, thus are not diagnostic.[1]

Serum PSA Test:

Prostate-specific antigen is a glycoprotein with a molecular weight of 34,000 Dalton. It is responsible for liquefaction of semen. PSA is highly specific for prostate tissue; it was first discovered when scientists were searching for a potential marker that could be used in investigation of rape crimes.

The PSA test is a blood test to measure levels of PSA in the serum. In the test, a serum sample is added to a tube containing two types of anti-PSA antibodies that recognize different antigenic sites on the PSA. One type of anti-PSA antibody is conjugated to an enzyme called alkaline phosphatase; the second type of anti-PSA antibody is conjugated to paramagnetic particles. If PSA present is present in the sample it binds to both antibodies forming a sandwich complex. A magnetic field is applied to separate the magnetic particles. The sample is washed to remove unbound alkaline phosphatase conjugate; thus the remaining alkaline phosphatase is proportional to the amount of PSA present in the sample. A chemiluminescent substrate called Lumigen PPD is added. Alkaline Phosphatase causes cleavage of the phosphate group on the Lumigen PPD producing an intermediate product. The intermediate product decomposes and generates chemiluminescence; this signal decays with a half life of several minutes. The light intensity produced is a direct measure of enzyme present.



PSA was first approved by the FDA in 1986 to monitor patients who had been treated for prostate cancer to determine whether they had a recurrence of disease. In the early 1990s physicians began to use the test to screen patient who were at risk for developing prostate cancer. Two large studies have been carried out to study the accuracy of the PSA test. When the cut-off value for an abnormal PSA test is set at 4 ng/L, its sensitivity has been reported to be 44-46%, with a specificity of 91-94%. The cost of a PSA test is approximately $50.

A number of approaches have been suggested to improve the sensitivity and the specificity of PSA based screening:

Adjust cut-offs with age since PSA levels increase with age.

Adjust cut-offs with ethnicity since African American males tend to have higher PSA values.

Monitor annual increases in PSA levels rather than absolute values.

Adjust PSA levels by the size of the prostate (PSA density).

Measure the fraction of free PSA relative to that bound to plasma proteins.

Sources: [65-70]

Prostate cancer is treated with a combination of surgery, radiation therapy, hormone therapy, and chemotherapy.[64] In the United States, the 5 year survival rate for all stages of prostate cancer combined is quite high at 99.9%. This is due to the effectiveness of treatments for cancer which is localized to the prostate, where the 5-year survival rate is 100%. When disease has metastasized to distant organs, the 5-year survival rate of prostate cancer is only 33.3%.[1] Thus, early detection of prostate cancer is important.

There are two tests which have been widely used to screen the general male population for prostate cancer, although there is considerable controversy regarding the most appropriate use of these tests. The first test is a simple blood test to measure levels of a protein called prostate specific antigen (PSA). PSA is a protein found on the surface of epithelial cells in the prostate. When prostate cancer develops, the number of epithelial cells increases and the amount of PSA found in the blood increases. A blood test can measure the levels of serum PSA quantitatively. However, other conditions which cause an increase in the number of prostate epithelial cells, such as benign enlargement of the prostate, can also cause PSA levels to be elevated. [64] The second test is to palpate the size of the prostate gland in a procedure called a digital rectal exam (DRE). The prostate gland lies close to the rectum, and its size can be felt by placing a gloved finger inside the rectum. Prostate enlargement can be a sign of either prostate cancer or benign prostate enlargement. [64] Screening using the PSA and DRE tests has become one of the most commonly used cancer screening tests. More than ½ of men over age 50 report having a recent serum PSA test and a digital rectal examination, although these figures drop to less than 33% for men without health insurance.[14]

If screening tests for prostate cancer are positive, further diagnostic tests to confirm or exclude the presence of prostate cancer are required. To confirm the presence of cancer, physicians obtain small pieces of prostate tissue called core needle biopsies. Biopsies are then sectioned, stained and observed under a light microscope to examine the epithelial cells of the prostate. A biopsy of prostate tissue is obtained by inserting a needle through the wall of rectum into the prostate (**Figure 10.40**). A positive screening test does not indicate where in the pros-

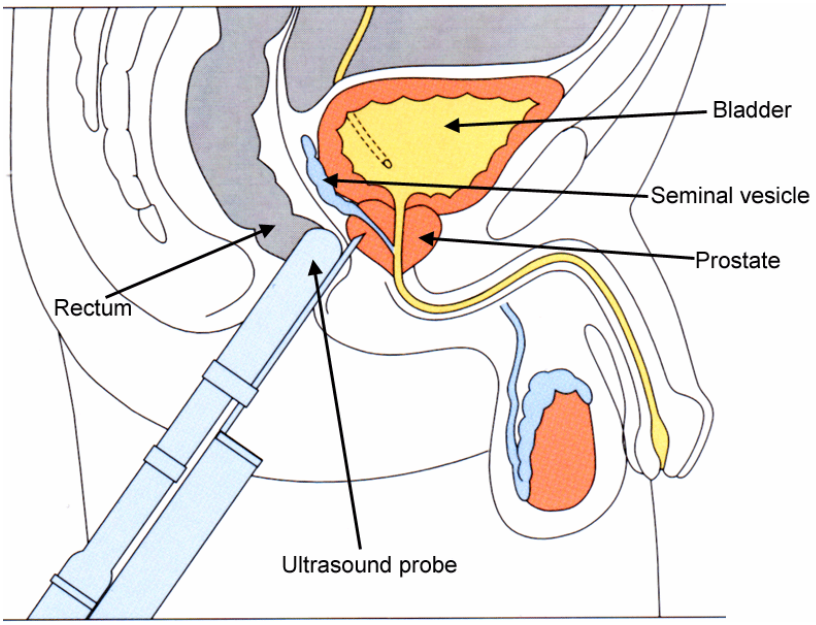


Figure 10.40: A diagram showing the prostate biopsy procedure.

Cancer Grade	Surgery 10-yr survival	Conservative 10-yr survival
Grade I	94%	93%
Grade II	87%	77%
Grade III	67%	45%

Table 10.12: The 10-year survival rates for three grades of prostate cancer following either surgery or conservative treatment. The earlier the cancer is detected, the less difference between survival rates for the two treatments.

tate a lesion might exist, so multiple biopsies are performed. Typically at least 10 core biopsies are obtained to sufficiently sample the prostate tissue; the procedure is performed with local anesthetic. The precise positioning of the needle is guided by ultrasound imaging. Small fragments of the prostate are then removed from the needle, processed, and examined under a microscope.[71] The cost of obtaining and processing a prostate biopsy is approximately $700.[70]

If prostate cancer is detected when it is still localized to the prostate, physicians generally recommend one of two courses of action. The first is radical prostatectomy, a surgical procedure to remove the prostate. While this procedure is usually curative, because it removes the cancerous cells, it has some very serious side effects. Because important nerves which control bladder function and sexual function are located in the same area as the prostate, they can be damaged during this surgical procedure.[64] Following radical prostatectomy, between 2-10% of men experience incontinence, and between 30-90% of men experience impotence.[11] Because of the seriousness of these side effects, other physicians recommend more conservative management of prostate cancer, choosing to watch the patient until symptoms develop, and then offering treatment.[64]

Because prostate cancer is a relatively slow growing cancer, there is some controversy over whether detection of very early disease makes a difference in patient outcomes. Localized prostate cancer is classified into 3 grades based on the severity of the disease. A study to examine the ten-year survival rates for localized prostate cancer found that the survival rates for surgery and conservative therapy were nearly the same for grade I disease, but were substantially higher when grade II or grade III disease were treated surgically (**Table 10.12**).[72]

This illustrates one of the challenges of screening for prostate cancer. Prostate cancer is a slow-growing cancer; the average patient does not show symptoms for an average of 10 years following the initial development of prostate cancer. Because prostate cancer occurs later in life, most men with prostate cancer actually die of other causes. For example, a 50 year old man has a 42% chance of developing microscopic prostate cancer sometime in his life, a 10% chance of having this cancer diagnosed, but only a 3% chance of dying of it.[73] As many as

Costs of Screening for Prostate Cancer:

We can examine the predictive value of the PSA screening test and the cost to find prostate cancer with this test. Let us assume that we test 1 million men between the ages of 50 and 59 for prostate cancer using a serum PSA test with a sensitivity of 44% and a specificity of 91%. The expected prevalence of prostate cancer is 10% in this population. The cost to screen is $50/patient, and a high serum PSA results in a follow up biopsy which costs $700. What are the positive and negative predictive values in this situation? What is the cost to screen the entire population? What is the cost to biopsy all men with positive tests? What is the cost/cancer found? To answer these questions, we fill in the 2x2 table below:

	Test Positive	Test Negative	
Disease Present	44,000	56,000	# with Disease = 100,000
Disease Absent	81,000	819,000	#without Disease = 900,000
	# Test Pos = 125,000	# Test Neg = 875,000	Total Tested = 1,000,000

The PPV and NPV are then PPV =44,000/125,000 = 35% and NPV =819,000/875,000 = 94%. Thus, a man with a negative PSA test has a 94% chance of not having prostate cancer. However, a man with a positive PSA test only has a 35% chance of having prostate cancer. The cost to screen the entire population is $50 million dollars. In addition 125,000 men will have a positive PSA test and require a biopsy. Note that 81,000 of these biopsies are unnecessary! The cost to biopsy this group is 81,000*$700=$56,700,000. Using this strategy we will find 44,000 cancers at a cost per cancer found of $56,700,000/44,000=$1,288.

Are the costs of screening with the PSA test a good use of health care resources? In Chapter 11, we will examine how to calculate the cost-effectiveness of different health interventions. Sources: [24, 69, 70]

20-50% of men who have died with no symptoms of prostate cancer have been found to have prostate cancer at autopsy. [69] Since the treatment of prostate cancer has significant side effects, patients and physicians are faced with difficult decisions about whether to treat the disease or watch the disease.

Thus, the question of whether to screen the general male population for prostate cancer has a complicated answer. Localized prostate cancer is curable, and advanced prostate cancer is fatal, indicating the benefits of detecting disease early through screening. While screening clearly has potential benefits, it also has potential risks. A positive screening results leads to a prostate biopsy, an expensive, reasonably invasive and uncomfortable procedure. For those patients whose screening test is falsely positive, this biopsy is unnecessary. Furthermore, because prostate cancer is such a slow growing cancer found in older men, screening may lead to over-detection of latent cancers. If we screen, we may detect many cancers that would never have produced symptoms before the patients died of other causes.

Let's examine some of this clinical evidence regarding the efficacy of screening. In Tyrol, Austria, the mortality from prostate cancer was constant from 1970-1993, prior to the introduction

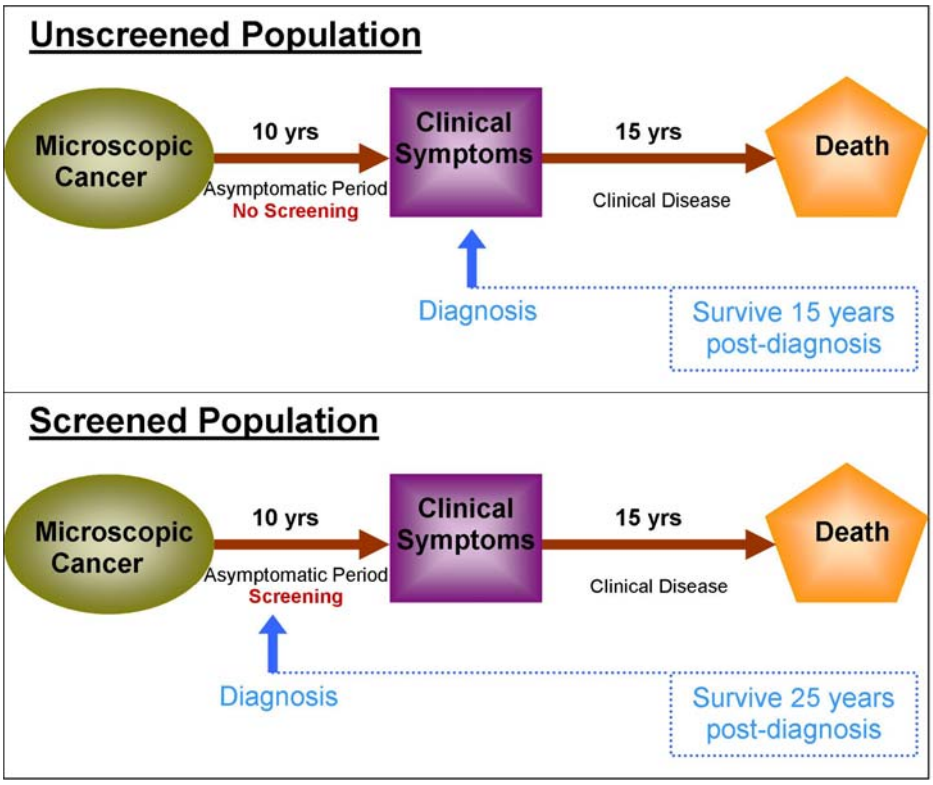


Figure 10.41: The natural history of prostate cancer versus time. The apparent increase in survival associated with screening is called lead time bias.

of mass screening. In 1993, mass screening for prostate cancer using digital rectal examination and serum PSA began.[74] Between 1993 and 2000, the mortality associated with prostate cancer decreased 44% in Tyrol.[75] While this study was not designed with a control group, cancer mortality remained constant in other parts of Austria where screening was not performed.

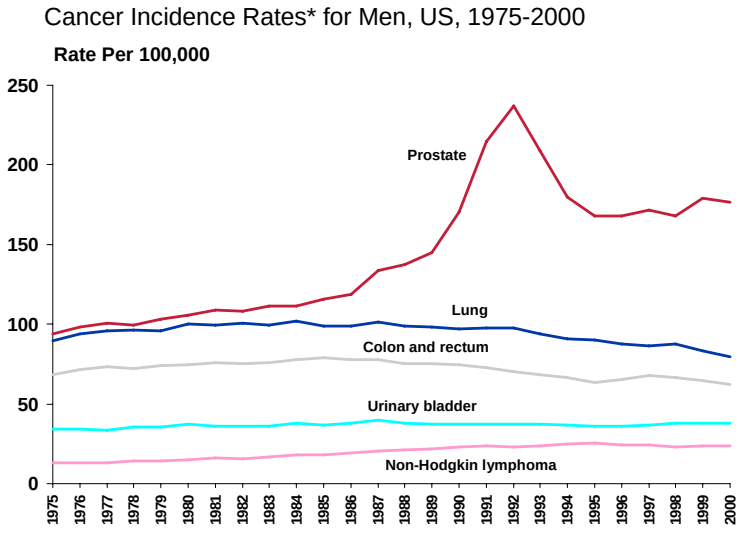
Other, more carefully designed and controlled studies have shown contrasting results. One completed randomized clinical trial of digital rectal examination and PSA to screen found no difference in the number of prostate cancer deaths between groups randomized to screening and usual care.[76] One prospective clinical trial in Canada suggested that screening with PSA could reduce the mortality due to prostate cancer by 67%, although the study was widely criticized for design and analysis flaws. Two large randomized clinical trials of screening are underway—the European Randomized study of Screening for Prostate Cancer (ERSPC) involving 200,000 men, and the Prostate, Lung, Colorectal, Ovarian cancer (PLCO) study involving 74,000 men in the US-but results are not expected to be available for years.[77]

The fact that prostate cancer is such a slowly growing cancer makes it difficult to perform a controlled experiment and test whether an intervention truly reduces mortality. **Figure 10.41** shows the natural history of prostate cancer vs. time. Once a

Organization	Recommendation
American Academy of Family Medicine	Physicians should counsel men between ages of 50 and 65 about known risks and uncertain benefits of screening so they may make an informed choice.
American Cancer Society	Offer the PSA and DRE tests annually beginning at age 50 to men who have a 10 year life expectancy and to younger men at higher risk
American College of Physicians	Physicians should describe potential benefits and known harms of screening, diagnosis an treatment, listen to patient's concerns and individualize the decision of whether to screen
American Urological Association	Men over 5 should consider testing. Men at high risk should begin testing at age 45.
CDC	Routine screening is not recommended because there is not consensus on whether screening and early treatment reduces mortality.
US Preventive Services Task Force	Evidence is insufficient to determine whether the benefits of screening outweigh the harms.

Table 10.13: There is no consensus on how, if, or at what age men should begin being screened for prostate cancer in the US [69].

Figure 10.42: Cancer incidence rates for men in the US vs. time. A large increase in prostate cancer incidence was reported shortly after initiation of PSA based screening in the late 1980s—early 1990s [21].



microscopic cancer develops, it typically takes 10 years before symptoms develop which would lead to a diagnosis even without the use of any screening tests. In this scenario, a typical patient survives 15 years beyond the initial diagnosis.[69] How does this sequence of events change if we screen for early disease? By screening asymptomatic patients, we detect disease earlier, as much as 10 years before symptoms develop. If our ability to detect prostate cancer early does not change the natural history of the disease, these screened patients do not live to be any older than patients who have not been screened. However, screened patients do survive for a longer period following diagnosis of their cancer, only because their cancer was detected before it produced clinical symptoms. This apparent increase in survival time following diagnosis is called 'lead time bias', indicating that the new intervention simply lead to earlier diagnosis without truly changing the outcome. Thus, randomized clinical trials must be carefully designed to minimize lead time bias.[78]

Given the limited clinical evidence currently available, different countries approach prostate cancer screening in different ways. In the United States, there are conflicting recommendations regarding screening (**Table 10.13**). The American Cancer Society recommends men aged 50 or older with more than a 10 year life expectancy should be screened with DRE and PSA. The American College of Preventive Medi-

	5-Year Survival, %			% Change (1950-1996)	
	1950-1954	1989-1995	Absolute Increase in 5-Year Survival, %	Mortality	Incidence
Prostate	43%	93%	50%	+10%	+190%
Cervix	59%	71%	12%	-76%	-79%
Ovary	30%	50%	20%	-2%	+3%

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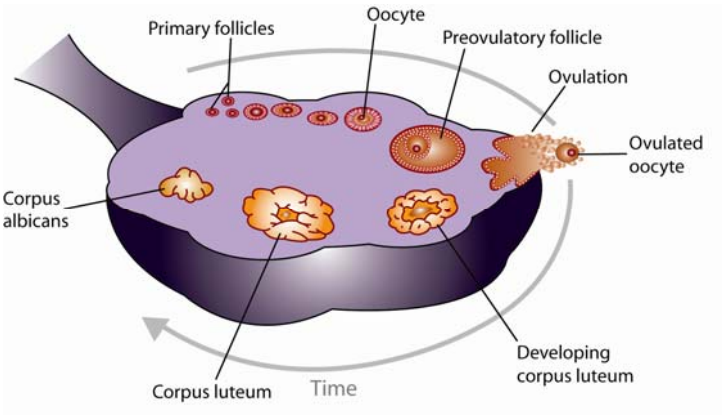
Table 10.14: Changes in survival rates and incidence for several cancer types since 1950 [79].

cine recommends that men aged 50 or older with >10 yr life expectance should be informed of the potential benefits and risks of screening and make their own decision.[69] The US Public Service Task Force in their Guide to Clinical Preventive Services, recommends against screening using DRE or serum PSA.[24] While they find good evidence that PSA screening can detect early-stage prostate cancer they conclude that there is mixed and inconclusive evidence that early detection improves health outcomes. They note that screening is associated with important harms, including frequent false-positive results and unnecessary anxiety, biopsies, and potential complications of treatment of some cancers that may never have affected a patient's health. They conclude that the available evidence is insufficient to determine whether the benefits outweigh the harms for a screened population. In Europe, screening is not currently recommended, because it is not believed that there is sufficient evidence to indicate that screening reduces mortality.[69]

How has the incidence and mortality of prostate cancer changed in the US following the introduction of screening? Routine screening has led to a dramatic increase in the number of cases of prostate cancer detected (**Figure 10.42**). The increase in incidence was accompanied by a shift in the stage at which prostate cancer is detected to earlier clinical stages. Over the period 1950-1996, the incidence of prostate cancer increased by 190% in the US (**Table 10.14**). Over this same period, the 5-year survival rate increased from 43% to 93%. However, the increase in 5-year survival rate may simply reflect the lead time bias associated with earlier detection. Over this same period, the mortality of prostate cancer in the US has actually increased by 10%. In contrast, during this same period cervical cancer screening led to a 79% decrease in the incidence of cervical cancer and a 76% reduction in the mortality of cervical cancer.

The next type of cancer we will consider is ovarian cancer; in contrast to cervical cancer and prostate cancer where screening tests are available, there is currently no good screening test for ovarian cancer. **Table 10.14** shows that the incidence and mortality of ovarian cancer have not changed appreciably from 1950-1996.[79]



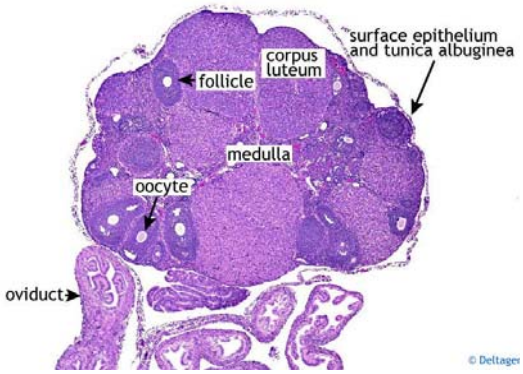


Figure 10.43: Left: Diagram of ovary indicating stages of ovulation. Right: Histologic photograph of ovary.

http://www.colorado.edu/kines/iphy4480tsai/ovary.jpg

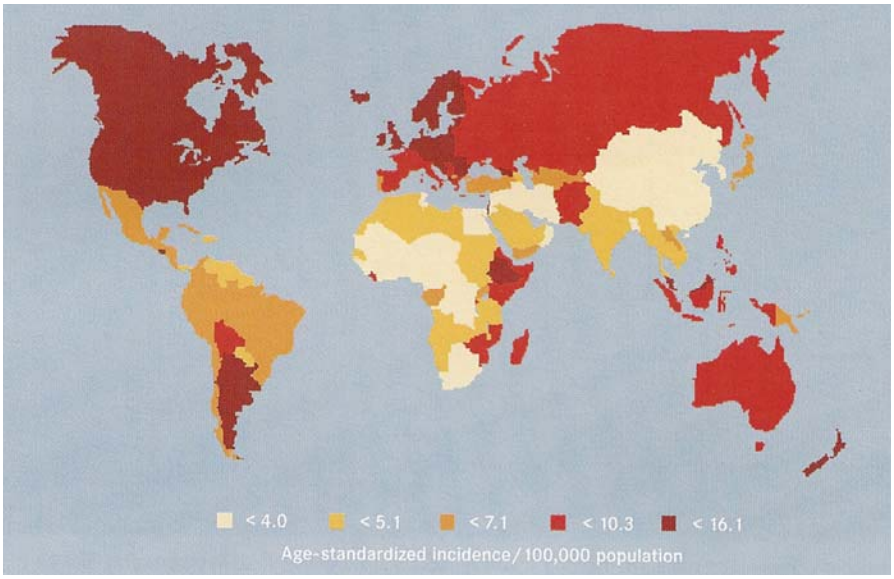
http://www.deltagen.com/target/histologyatlas/atlas_files/female_rep/ovary_4x.htm

Ovarian Cancer: We have considered two cancers where screening tests are available: cervical cancer and prostate cancer. In our final example, we turn to a cancer where there is no adequate screening test – ovarian cancer. The ovaries are part of the female reproductive system (**Figure10.43a,b**) and are located adjacent to the fallopian tubes. In the US in 2007, there will be an estimated 22,430 new cases of ovarian cancer, representing 3.3% of all cancers in women.

An estimated 15,280 women will die as a result of ovarian cancer in 2007 in the US.[1] Worldwide there were 190,000 new cases of ovarian cancer and 114,000 deaths in this same year. The highest rates of ovarian cancer occur in Scandinavia, Eastern Europe, USA, and Canada (Figure 10.44).[11]

The treatment for ovarian cancer involves surgery, and for advanced disease involves radiation therapy and chemotherapy. The 5-year survival rate for all stages of ovarian cancer is 45%.[1] There are four stages of ovarian cancer; when detected early, the 5-year survival rates are much higher. 90% of women diagnosed with stage I ovarian cancer, when the disease is localized to the ovaries, survive 5 years beyond their initial diagnosis. However, the 5-year survival rate for metastatic, stage III-IV ovarian cancer is only 25-37%.[80] Unfortunately, because of the lack of good screening tests and the fact that early ovarian cancer produces relatively few symptoms, more than 70% of women diagnosed with ovarian cancer are diagnosed at stages III and IV.[1] **Table 10.15** compares the ratio of mortality rate to the incidence rate

Figure 10.44: Global incidence rates of ovarian cancer.



for the ten most common cancers in women; ovarian cancer has one of the highest mortality to incidence ratios, second only to pancreatic cancer and lung cancer.[81] On average, women who die of ovarian cancer lose 18 years of life to the disease.[82]

Site	Incidence*	Mortality*	Mortality:Incidence Ratio
Breast	127.8	25.5	0.20
Lung & Bronchus	52.3	41.1	0.79
Colon & Rectum	44.6	16.4	0.37
Uterine Corpus	23.2	4.1	0.18
Non-Hodgkin Lymphoma	16.3	6.2	0.38
Melanoma of the Skin	14.9	1.7	0.11
Ovary	13.5	8.9	0.66
Thyroid	12.5	0.5	0.04
Pancreas	10.1	9.2	0.91
Urinary Bladder	9.4	2.3	0.24

* Age-adjusted rate per 100,000 women per year; based on US cases diagnosed in 200-2004

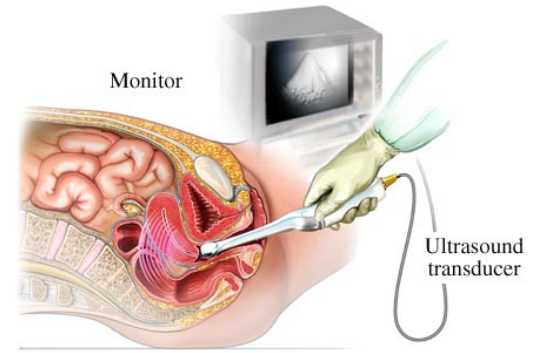
Table 10.15: Mortality/incidence ratio in ten most common solid cancers in women in the US.

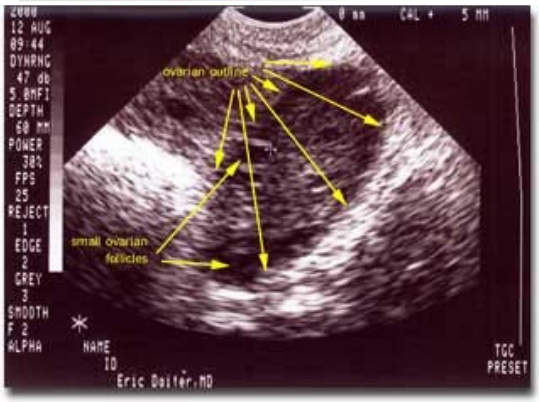
From Rosenthal et al, Clinical Obstet and Gynecol, 49 (3)433-447, 2006.

Ovarian cancer is said to "whisper" because the symptoms are so vague. Symptoms can include unexplained change in bowel and/or bladder habits, such as constipation, urinary frequency, incontinence; gastrointestinal upset, such as gas, indigestion, nausea; unexplained weight loss or weight gain; pelvic and/or abdominal pain or discomfort, bloating or swelling; a constant feeling of fullness; fatigue; abnormal or postmenopausal bleeding and pain during intercourse. Frequently, women (and their physicians) will attribute these symptoms to those normally experienced with aging.

There are a number of factors that put a woman at higher risk for developing ovarian cancer. The most important risk factors are a personal or family history of breast, ovarian, endometrial, prostate or colon cancer, particularly having one or more first-degree relatives (mother, sister, daughter) who have ovarian cancer. Ovarian cancer is sometimes associated with a mutation in the BRCA1 or BRCA2 gene. Hereditary ovarian cancer accounts for about 10% of cases.[80] In addition, the risk of ovarian cancer increases with the more lifetime cycles of ovulation that a woman has undergone. Thus, women who have undergone hormonal treatment for infertility, never used birth control pills, and who never became pregnant are at higher risk for ovarian cancer. In addition, the use of high dose estrogen for long periods without progesterone may also increase the risk of developing ovarian cancer.

The ovary is an almond shaped organ that contains all the eggs that will be released over a woman's reproductive lifetime (**Figure 10.43a,b**). The ovary is lined by a single layer of epithelial cells. Beneath the epithelium, the ovary contains spherical follicles, each containing a single oocyte (egg), in a region known as the ovarian cortex. At the very center of the ovary, blood vessels bring in oxygenated blood and nutrients in





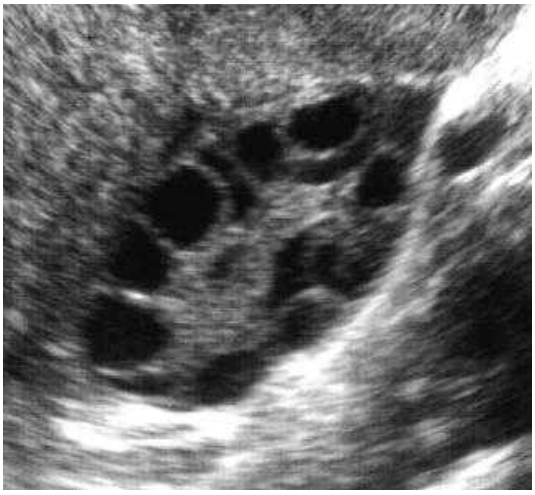


Figure 10.44 a,b,c: Transvaginal ultrasound can be used to image the ovary.

http://www.memorialhermann.org/library/healthguide/en-us/images/media/medical/hw/nr551775.jpg

http://www.ivf-infertility.com/images/polycystic_ovary.jpg

http://www.infertilitytutorials.com/images/transvaginal_ultrasound.jpg

a region known as the ovarian medulla. Each month, one or more follicles undergoes a transformation in preparation for ovulation. A primordial follicle enlarges and develops into a primary follicle. The follicle continues to enlarge and move toward the surface of the ovary. The secondary follicle then merges with the ovarian surface, ruptures and releases the oocyte. The defect in the ovarian surface must then repair itself. The scar left behind is known as a corpus albicans. Thus, the surface of the ovarian epithelium is constantly undergoing damage and repair. During this process, epithelial cells can become transformed and lead to ovarian cancer. As the frequency of this repair process increases, so do the chances that an ovarian epithelial cell will become transformed leading to an ovarian cancer. This probably explains why the use of oral contraceptives, pregnancy and breast feeding reduce the risk of ovarian cancer development.

Because ovarian cancer does not generally produce symptoms until very advanced stages, there has been substantial research to develop good early detection tools. Three are available, but all suffer from significant limitations; these techniques include: (1) pelvic and rectal examinations, (2) the CA-125 blood test, and (3) transvaginal ultrasound.

Pelvic and rectal examinations are normally conducted when a woman has a Pap smear. In this procedure, a physician manipulates the abdomen to feel the uterus and ovaries to find abnormality in shape or size. While this procedure can detect large changes associated with advanced ovarian cancer, it is unlikely to detect early stage ovarian cancer.

The CA-125 blood test is similar to the use of PSA to screen for prostate cancer. Ovarian cancer cells produce a protein called CA-125 which is released into the blood stream. 80% of women with advanced ovarian cancer have elevated CA125 levels.[83] In fact, physicians routinely used blood levels of CA-125 to monitor women following treatment for ovarian cancer – it is a sensitive indicator of persistent or recurrent disease.[84] Unfortunately, CA-125 levels are very unreliable for detecting early cancer, particularly in pre-menopausal women. The reasons are two fold: first, CA-125 levels are often not elevated in early ovarian cancer. Second, CA-125 levels can be elevated by conditions such as pregnancy, endometriosis, uterine fibroids, liver disease, and benign ovarian cysts.[85] Thus, in a pre-menopausal woman, an elevated CA-125 level is much more likely due to a benign cause than due to ovarian cancer.[81] The sensitivity and specificity of serum CA-125 levels in one large Norwegian study were an overall sensitivity of 30-35%, with a specificity of 95.4%.[86, 87] In general, the sensitivity is lower for detecting early stage disease.

Finally, ultrasound imaging can be used to visualize the ovaries. It is difficult to use ultrasound to visualize the ovaries through the abdominal wall. In order to view the small ovaries, an ultrasound probe is inserted into the vagina, and placed close to the ovaries. Using high-frequency sound a picture of the ovaries is created (**Figure 10.44a,b,c**). Transvaginal ultrasound can detect ovarian malignancies in asymptomatic women, based on the increase in ovarian volume, and the presence of complex cysts within the ovary.[88] However, it has poor accuracy in detecting early stage disease. A recent large study of transvaginal ultrasound to screen 14,469 asymptomatic women achieved a sensitivity of 81% and a specificity of 98.9% for the detection of ovarian cancer.[89]

Improved CA-125 Tests:

Recent attempts to improve the performance of screening using CA-125 have focused on using an algorithm that incorporates patient age, absolute levels of CA-125 and the rate of change of CA-125. Using this approach, a sensitivity of 83% and a specificity of 99.7% have been achieved.
Source: [81].

The only way to confirm a positive screening test for ovarian cancer is to perform a biopsy of the ovary. Because the ovaries are located in the abdominal cavity, this procedure involves surgical exploration of the abdomen to visualize and potentially biopsy the ovaries. Typically, this surgery is performed through a laparoscope (**Figure 10.45**). As we will see in detail in Chapter 14, in this procedure a small trochar is punched through the abdominal wall and the abdomen is inflated with CO2 gas. Then, fiber optic laparoscopes are inserted through the abdomen to view the ovaries; small biopsy forceps can also be inserted to sample the tissue; a diagnosis of ovarian cancer can be definitely made by examining the biopsy in the same way that a cervical biopsy is examined. Approximately 1% of women undergoing laparoscopy will have a complication that will require an open surgical procedure.[90]

Let's consider what happens when we screen a group of women for ovarian cancer using the available screening and diagnostic tests. If we screen 1,000,000 women in a setting with a 0.03% prevalence of undiagnosed ovarian cancer, there are a total of 300 cases that we can possibly detect.[91] Let's assume we use the CA125 blood test to screen our patients, and recommend that those women with an elevated CA125 have a laparoscopy. The sensitivity of CA125 is 35% and the specificity is 95.4%.[86] The test costs about $60 to perform.[92] In this scenario, we will spend $60 million to screen our population; the

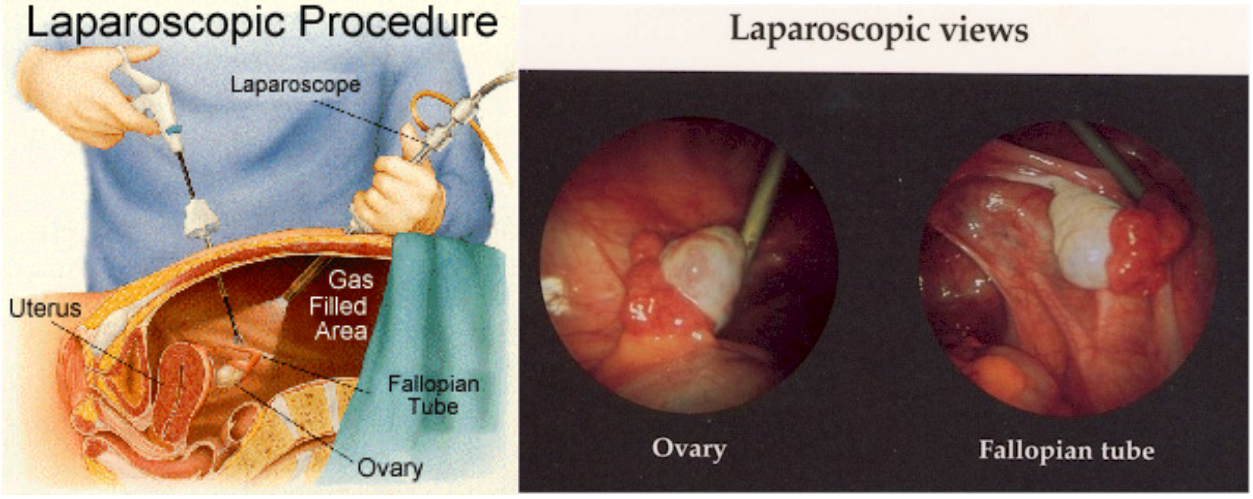


Figure 10.45: An ovarian biopsy is obtained during laparoscopy. A fiber optic catheter is used to visualize the ovaries and guide biopsy direction.

http://www.aiof.com/html/images/lapro.jpg

screening test will identify 105 true positives, and a staggering 45,986 false positives, all of whom will undergo laparoscopy and biopsy, which is our gold standard. The cost of laparoscopy is approximately $1,500 and 1% of women undergoing laparoscopy will suffer a serious complication requiring open surgery.[90, 93] In this scenario, we will spend $1,229,871 for each cancer that we find. Although we find only 105 cancers, 195 cancers will go undetected and 45,986 women will undergo an unnecessary laparoscopy and 460 women will suffer a complication as a result. In this scenario, the number of patients who suffer a serious complication caused by screening (460 women) exceeds the number of women correctly diagnosed with ovarian cancer (105 women). The PPV of this screening strategy is only 0.23%, the NPV is 99.98%.

If we use transvaginal ultrasound to screen our same population, the outcomes improve somewhat. The sensitivity of transvaginal ultrasound is 81% and its specificity is 98%.[91] The cost to perform this imaging procedure is approximately $200.[92] In this scenario, we identify 243 of the 300 ovarian cancers, but 19,994 false positives lead to unnecessary laparoscopies, resulting in 202 complications. While the cost to detect a case of ovarian cancer is reduced to $947,965 in this strategy, the associated PPV is still dismally low at 1.2%; the NPV is 99.99%.

We can examine the use of transvaginal ultrasound in a population with a higher prevalence of ovarian cancer. If we screen post-menopausal women over the age of 45, the prevalence of undiagnosed ovarian cancer rises to approximately 0.2% .[94] In our cohort of 1,000,000 women, there are 2000 cases of ovarian cancer. In this population, transvaginal ultrasound correctly identifies 1,620 women with ovarian cancer. The approach results in 19,960 false positives, and 216 serious complications. The cost to detect a single case of ovarian cancer is reduced to $143,438, and the PPV is 7.51%.

In this population, how high does the specificity of our screening test need to be in order to achieve a PPV of 10%? A simple calculation shows that the test specificity must reach 99.9% in order to achieve even a modest PPV, where 1 in every 10 follow up laparoscopies will identify an ovarian cancer. This illustrates the difficulty of screening for a rare disease – in general, unless the specificity of the test is extremely high, the number of false positive results will far exceed the number of true positive results. If the follow-up test carries any risk, then screening for a rare disease can actually cause greater harm than good.

Let's examine what has happened in an actual clinical trial of these technologies to screen for ovarian cancer. The most successful results have been obtained using a combination of approaches to screen for ovarian cancer. A randomized clinical trial of 22,000 women compared no screening to a combination of screening with CA-125 and transvaginal ultrasound.[95] In the group of women who were screened, CA-125 blood tests were performed annually for three years. If the CA-125 lev-

els exceeded a threshold level, transvaginal ultrasound was performed. In the screening group of 10,958 women, 468 women underwent 781 ultrasound exams because their CA-125 levels were elevated. 29 women underwent biopsy to detect 6 cancers. Thus, the overall positive predictive value of multi-modal screening was 6/29 = 20.7%. While the predictive value of this approach is higher, there are concerns that the sensitivity of this approach is not high enough. Despite the screening provided in this study, an additional 10 women in the screening group developed ovarian cancer during a follow up of 8 years. Five of the 16 cancers discovered in the screening group were stage I or II, whereas only 2 of the 20 cancers discovered in the control group were stage I or II.

Because ovarian cancer is such a devastating disease, there are a number of ongoing trials testing new screening approaches.[81] In the UK, a trial of 200,000 postmenopausal women is underway, comparing annual screening with CA 125 or transvaginal ultrasound to no screening .[96] Results are expected in 2012. In the US a trial of 78,000 is underway comparing the ability of annual serum CA 125 and transvaginal ultrasound to no screening.[97] Results of these clinical trials will help determine future screening recommendations throughout the world.

New Screening Tests for Ovarian Cancer: Because of the limitations of current screening tests, researchers are searching for additional markers that might be useful for ovarian cancer screening. Most current cancer screening tests look for a single protein in the serum (e.g. CA-125, PSA). However, serum contains many proteins; it may be possible to identify complex patterns of serum proteins which are predictive of cancer. This field is called proteomics. In this approach, researchers use techniques to analyze the patterns made by all proteins in the blood, without even knowing what they are.

The technique used to measure the pattern of serum proteins is known as mass spectrometry. In this technique, serum proteins are extracted, and bombarded with an electron beam. The electron beam has sufficient energy to fragment the proteins. This process produces charged fragments, most of which have a unit positive charge. These tiny charged fragments are then sprayed out of a nozzle through a magnetic field into a vacuum chamber. The positively charged fragments are accelerated in the vacuum chamber through a

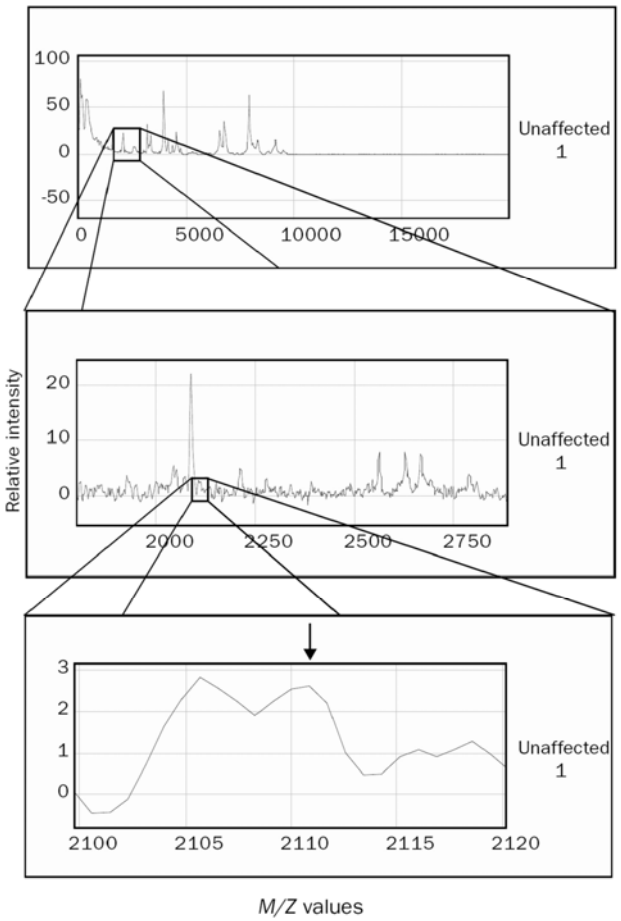


Figure 10.46: Mass spectrometry of blood serum may help differentiate healthy individuals from those with cancer [83].

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strong magnetic field. The time required for each fragment to travel down this chamber is dependent on the ratio of its mass to charge. The mass spectrometer produces a graph that shows distribution of masses in the sample. A computer program is then used to analyze patterns and distinguish blood from patients with cancer and from those without.

Figure 10.46 shows a typical mass spectrograph. The protein fragment mass is indicated on the x-axis, while the strength of the signal plotted on the y axis is proportional to the amount of protein fragment with that mass in the sample. If one does mass spectrometry using a chemically pure sample, the mass of each fragment of the molecule enables one to determine the chemical structure of the sample, by working backwards to generate the original molecule. This technique is frequently used by chemists to identify the structure of an unknown chemical compound. However, serum contains a mixture of many proteins, with widely varying concentrations. In this case, instead of a series of a few sharp peaks, the resulting mass spectrum contains many peaks, of varying height. While one cannot use these data to work backwards and reconstruct the structure of each protein, it can be used to identify patterns of proteins that differ between healthy and diseased patients.

Do you think it is fair to compare the PPV of this test in this setting to our PPV calculations for CA125 and transvaginal ultrasound? Why or why not?

Recently a new blood test based on this technique to screen for ovarian cancer received widespread media attention. The test was first described in the medical literature in 2002.[83] In this test, a blood sample is obtained from a patient. Serum proteins are isolated and the sample is analyzed using mass spectrometry. Scientists obtained blood from 50 women known to have ovarian cancer, 50 women known to be normal and 16 women with benign ovarian disease. They analyzed the resulting mass spectrometry data to search for protein peaks which differed in these two groups of patients. They examined thousands of proteins and identified a few which appeared to be different in the two groups.

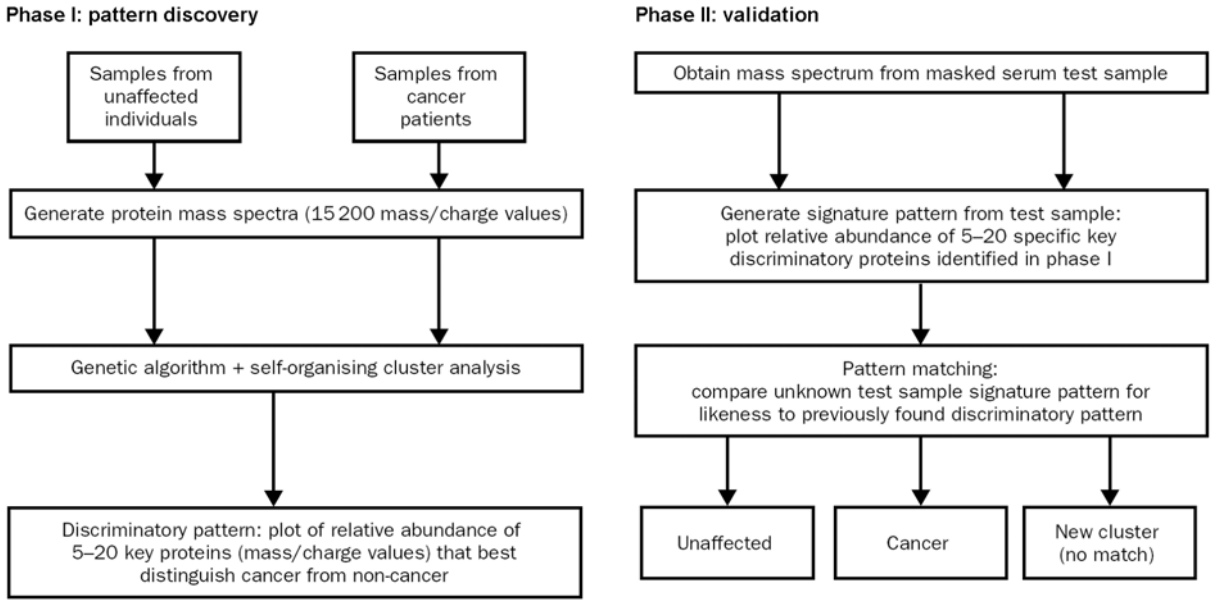

Figure 10.47: The initial phase in developing the blood test to screen for ovarian cancer (**a**) and the subsequent phase, validating the pattern found in the first phase (**b**).

Using these differences they were able to define a diagnostic algorithm which correctly identified 50 out of 50 patients with ovarian cancer (sensitivity = 100%), and correctly identified 63 out of 66 women as normal (specificity = 95%). You can easily show that, in this setting, the positive predictive value of this test is 94%, significantly higher than what we calculated for CA125 or transvaginal ultrasound.

Let's examine the development of this test in more detail. In the initial phase of the study, called pattern discovery (**Figure 10.47a**), blood samples were obtained from patients known to have cancer and patients known to be normal. Protein mass spectra were obtained from each of these samples, and investigators examined the spectra. Each one contained the strength of the signal at 15,200 different mass/charge ratios. Different types of data analysis were applied to identify a small group of 5-20 key proteins which differed between the two groups of patients. The proteins were characterized by their mass/charge ratio and their relative abundance. This phase of the study is sometimes called the training phase, because it focuses on narrowing down a large number of data points, to identify a small group which provide diagnostically useful information. However, one limitation of this approach is that the number of proteins measured usually greatly exceeds the numbers of patients participating in the trial. Under these conditions, it is possible that differences in protein abundance between patients with cancer and patients without cancer arise due to simple chance fluctuations, and have nothing to do with the disease process at all.[83]

From the thousands of peaks measured, the abundance of protein at only 5 different mass to charge ratios was found to vary between patients with and without cancer. **Figure 10.48** shows data from 4 patients – two with cancer and two without. There is a very different mass to charge ratio at 2111 in the spectra from cancer patients compared to the spectra from unaffected individuals. To guard against the possibility that these fluctuations are due to chance, most clinical trials to test new diagnostic tests use a training phase to optimize the algorithm. Then, a second group of patients is recruited and the diagnostic algorithm is applied to data collected from this group; a phase generally referred to as validation (**Figure 10.47b**). The performance of the diagnostic algorithm when applied to data in this validation group gives the best estimate of how well the algorithm performs.[83]

Accounts describing the exciting promise of this new diagnostic test were widely reported in the media (see short article from the February 18, 2002 issue of Newsweek above). At the time, the lead author on the study, Lance Liotta, said, "The most important next goal is validating the promise of these results in large, multi-institutional trials."[98] While the general media responded with enthusiasm to the possibility of a new test which could improve the early detection of cancer, response from the scientific community was much more skeptical. Dr. Eleftherios P. Diamandis, head of clinical biochemistry at Mount Sinai Hospital in Toronto, expressed the concern that, "If you don't know what you're meas-

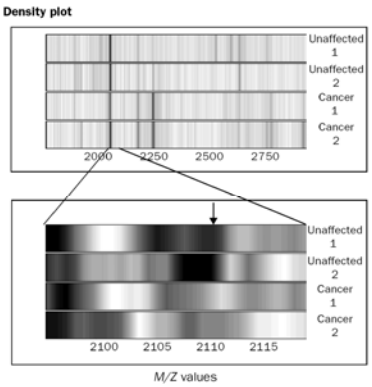

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Figure 10.48: Spectra illustrating that a difference in mass to charge ratio may differentiate patients with ovarian cancer from those without it [83].

Read More About It:

Results of the new, proteomic diagnostic test for ovarian cancer are widely reported in the popular press:

Underwood A. Testing: Ovarian Cancer. Newsweek. 2002 February 18: pg12.
Source: [100].

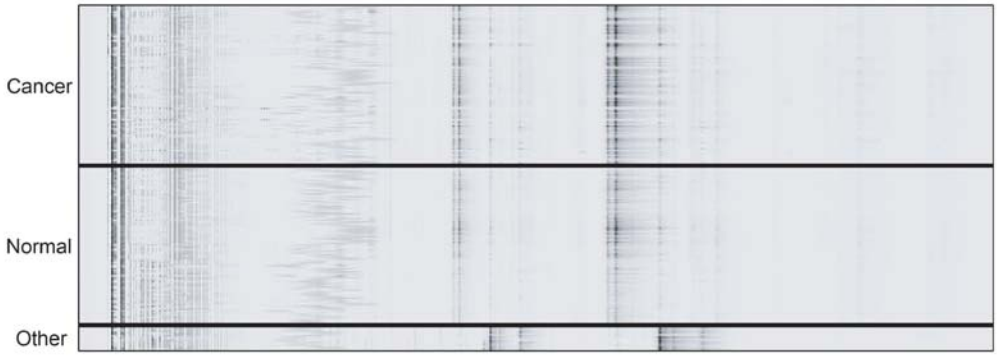

Figure 10.49: A heat map representation of all the ovarian cancer screening study specimens; the shifted spectra at the bottom raise questions about the experimental protocol of the study [101].

uring, it's a dangerous black-box technology… They are rushing into something and it could be a disaster."[99] Dr. Nicole Urban, head of gynecologic cancer research at the Fred Hutchinson Cancer Research Center in Seattle warned patients, "Certainly there's no published work that would make me tell a woman she should get this test."[99]

The datasets used to generate the ovarian cancer screening algorithm were made publicly available. When others tried to reproduce the results reported in the literature, several problems were identified. Most importantly, it appeared that there was a change in the experimental protocol for the measurements made from benign specimens that caused a systematic change in the data. **Figure 10.49** shows a heat map representation of the 216 spectra from the pattern discovery and validation phases of the data. The m/z ratio runs along the x-axis, and the samples are grouped by diagnosis. There is a clear difference in the pattern of the benign specimens shown at the bottom of the figure possibly due to a change in protocol for these specimens.[101]

The use of proteomics technology at present can be thought of as a 'black box technology'. Serum samples are sent into the black box, and a diagnosis comes out. Because the approach does not rely on biological explanations, it is crucial that the approach be reliable and reproducible in any location. Further studies with additional samples are required to demonstrate the potential of this new technology.

Summary: In this Chapter, we have seen the benefits and possible harms associated with cancer screening. Screening should be undertaken only when the following conditions have been met: (1) the effectiveness of the screening test has been demonstrated, (2) there are sufficient economic resources to screen all patients in the target group, (3) there are tools to confirm disease in patients with a positive screening test, (4) there are existing procedures to treat the disease, (5) and when disease prevalence is high enough to justify effort and costs of screening.

One of the challenges of screening is that it may reveal disease that might never be detected or cause problems otherwise. This is certainly true for screening for cervical cancer with the Pap test. Most abnor-

malities found on the Pap smear never become invasive cancer. However, there are relatively low-cost, minimally invasive tools to follow an abnormal Pap smear, and treatment of high grade precancer can prevent future development of cervical cancer. The use of screening has dramatically reduced both the incidence and the mortality of cervical cancer throughout the developed world. Likewise, screening for prostate cancer likely identifies many cases of prostate cancer which would otherwise have never produced any symptoms. Unlike the case of cervical cancer, screening with the PSA test has dramatically increased the apparent incidence of prostate cancer, while the mortality has largely remained unchanged. Ovarian cancer presents one of the most difficult challenges in cancer screening; because it is a relatively rare disease, any potential screening test must have a very high specificity to yield a reasonable predictive value. The relative inaccessibility of the ovaries makes it difficult and invasive to follow up an abnormal screening test. As a result, we do not currently screen for ovarian cancer although it is the most deadly of the female reproductive cancers.

While screening can have important medical benefits, it requires resources, both to test people and to follow up abnormal screening results. How do we decide if screening represents a good investment of health care resources? In the next chapter, we will examine the use of cost-effectiveness analysis to make these decisions.

Bioengineering and Global Health Project

Project Task 6: Gather information regarding current research and development efforts.
What research and development efforts are currently underway to solve the health problem that you have identified? Write a one-page summary of this research, summarizing what is known about the effectiveness or limitations of these current procedures.

Chapter 10 Homework

1. In the U.S., what is the most prevalent cancer in (a) men and (b) women? Worldwide, what is the most prevalent cancer in (c) men and (d) women?

2. Cancer screening:

a. What four types of cancer are routinely screened for in the United States? For each, describe the screening test that is used.
b. Do most people in the US adhere to screening recommendations? What factors cause people not to be screened?
c. Discuss whether these screening tests are used throughout the rest of the world.

3. Lung cancer is the leading cause of cancer death for both men and women in the United States. More people die of lung cancer than of colon, breast, and prostate cancers combined. Lung cancer is fairly rare in people under the age of 40. The average age of people found to have lung cancer is 60. In 2004 there will be about 173,770 new cases of lung cancer in the United States. About 160,440 people will die of this disease. The population of the United States in 2004 is 292,287,454.

a. Calculate the annual incidence rate of lung cancer in the US in 2004.
b. Calculate the mortality rate of lung cancer in the US in 2004.
c. Why is the mortality rate of lung cancer so high?

4. Describe in your own words, ***WITHOUT*** using equations or other mathematical expressions or the words “true”, “false”, “positive”, or “negative” the following terms with regard to a screening test for ovarian cancer:

a. True Positive
b. False Positive
c. False Negative
d. True Negative
e. PPV
f. NPV

5. A diagnostic test is 92% sensitive and 94% specific. A test group is comprised of 500 people known to have the disease and 500 people known to be free of the disease. How many of the known positives would actually test positive? How many of the known negatives would actually test negative?

6. A screening test for a particular disease has a sensitivity of 96% and a specificity of 92%. You plan to screen a population in which the prevalence of the disease is 0.2%. How many false positives will be found by this screening procedure for each true positive that is found?

7. A clinical trial of a new automated mammography system was carried out in 50,000 women known to have breast cancer. If 37,500 women received a positive test result, what would the specificity of the new test be?
8. Based on all the information currently available, you estimate that the patient in your office has a one in four chance of having a serious disease. You order a diagnostic test with sensitivity of 95%

and specificity of 90%. The result comes back positive. Based on all the information now available, what would be the chance your patient really has the disease?

9. A test with 99.9% sensitivity and 99% specificity is used to screen a population for a disease with 1% prevalence. What would be the proportion of test positives in the screen who actually have the disease?

10. The American Disease X Foundation reports that 6% of the population over 50 years of age has Disease X. You inquire as to the source of their information, and they cite disease population screening data in the literature which reports that 6% of that population was positive when screened. Referring to the literature, you discover that the screening test used had sensitivity of 95% and specificity of 98%. What proportion of the population over 50 years of age do you think really has the disease?

11. A recent study examined the expression of p53 (a protein found in many transformed cell lines derived from tumors) as a marker for ovarian cancer. The sensitivity and specificity of p53 as a marker for the diagnosis of ovarian cancer in this study were 82% and 93% respectively. Forty-seven patients with no family history of breast or ovarian cancer were included in the study. Fourteen of the 17 patients with ovarian cancer had p53 overexpression. Fifteen of the 47 patients had never given birth.

a. If p53 overexpression was used as a test for ovarian cancer, how many patients in this study received a false positive test result?
b. If p53 overexpression was used as a test for ovarian cancer, how many patients in this study received a false negative test result?
c. How much better are these results for a screening test than CA-125?

12. You are a physician for Mr. Jones, a 65 year old African American man who presents to you with complaints of difficulty urinating. Specifically, he has trouble starting urine flow and has an intermittent stream. He says he noticed this problem some time ago, and that it has slowly been getting worse. Mr. Jones says he has always been healthy and has not seen a doctor in thirty years. He was adopted and does not know his family history.

a. What disease discussed in Chapter 10 might explain Mr. Jones' symptoms?
b. What three risk factors for this disease does Mr. Jones have?
c. What initial tests are available that might aid your diagnosis of Mr. Jones?
d. If the initial tests are positive, what would be the next step in diagnosis?
e. Mr. Jones does indeed have the disease you suspected, and you recommend surgical intervention. Any surgical procedure has the risks of pain, bleeding, and infection. What are two specific risks associated with this particular surgery?
f. List two reasons why the tests listed in part c are controversial for use as screening tools.
g. In American males, prostate cancer is the most common, non-skin cancer (accounting for 33% of all new cancers), but is less deadly than might be expected, ranking behind both lung and colon cancer as the third leading cause of cancer death (9%). By contrast, ovarian cancer is the eighth most common new non-skin cancer in American women (3% of new diagnoses), but accounts for a surprising number of deaths; it ranks as the fifth leading cause of cancer death in this population (6%). Give three reasons for the discrepancy between the incidence and death rates for these two cancer types.

13. A patient comes to your office complaining of abdominal fullness and a change in bowel habits. She reports a family history of breast cancer and ovarian cancer. You suspect she may have ovarian cancer and order a serum CA125 test. The sensitivity of this test is 35% and the specificity is 98.5%. The incidence of ovarian cancer in this population is 0.1%. The test comes back positive.

a. If you gave this test to 1,000,000 women, how many patients would have a true positive (TP) result, a false positive (FP) result, a true negative (TN) result and a false negative (FN) result?
b. Given her positive test result, what is the likelihood that your patient really has ovarian cancer?
c. What test would you recommend that your patient undergo next?

14. A company called BioCurex recently announced results of a clinical trial for a new test to detect lung cancer (see story below).

RANCHO SANTA MARGARITA, Calif.--(BUSINESS WIRE)--April 5, 2004--BioCurex Inc. announces results for lung cancer detection using its proprietary Serum-RECAF(TM) blood test. The results confirm 90% sensitivity with 95% specificity. The findings further substantiate the use of RECAF(TM) as a universal cancer marker with a potential market size of $2 billion per year for all cancers. The study included 32 lung cancer patients and 103 normal donors with statistical verification.

[http://www.biospace.com/ccis/news_story.cfm?StoryID=15650520&full=1]

a. Calculate the number of patients with true negative (TN), true positive (TP), false positive (FP) and false negative (FN) test results in this trial.
b. What is the positive predictive value in this trial?
c. Do you think the PPV you calculated in part b is an accurate estimate of what to expect if the test is used to screen the general population for lung cancer? Why or why not?

15. Suppose we have two new screening tests for ovarian cancer – Test A and Test B. When tested in a large population, we find the sensitivity and specificity values for the two tests listed in the table below. Your mother knows that you have taken BME301. She is worried about her risk of ovarian cancer because both her mother and sister died of ovarian cancer at a young age. She asks your advice about which screening test to undergo. Which test would you recommend that she take? Why?

Test	Se	Sp
Test A	60%	95%
Test B	95%	60%

16. Consider the development of a new proteomics based screening test for ovarian cancer described in this chapter. Apply the five steps of technology assessment to this new technology. Does this assessment support the use of the technology?

References

[1] American Cancer Society. Cancer Facts and Figures 2007. Atlanta: American Cancer Society; 2007.
[2] Miniño AM, Heron MP, Smith BL. Deaths: Preliminary Data for 2004. National Vital Statistics Report. 2006 June 28;54(19).
[3] American Cancer Society. Cancer Facts and Figures 2005. Atlanta: American Cancer Society; 2005.
[4] Edwards BK, Brown ML, Wingo PA, Howe HL, Ward E, Ries LAG, et al. Annual Report to the Nation on the Status of Cancer, 1975 – 2002, Featuring Population-Based Trends in Cancer Treatment. Journal of the National Cancer Institute. 2005 October 5;97(19).
[5] WHO. Mortality: Revised Global Burden of Disease (2002) Estimates. Geneva: World Health Organization; 2002.
[6] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA: a cancer journal for clinicians. 2005 Mar-Apr;55(2):74-108.
[7] International Union Against Cancer. Global Action Against Cancer Geneva: WHO/UICC; 2005.
[8] World Health Organization. Global cancer rates could increase by 50% to 15 million by 2020. Geneva: World Health Organization; 2003 April 3.
[9] American Cancer Society. The Case for Global Action. 2007 [cited 2007 May 9]; Available from: http://www.cancer.org/docroot/AA/content/AA_2_5_1x_Case_for_Global_Action.asp?sitearea=AA
[10] American Cancer Society. Lifetime Probability of Developing or Dying from Cancer. 2006 February 22 [cited 2007 May 9]; Available from: http://www.cancer.org/docroot/CRI/content/CRI_2_6x_Lifetime_Probability_of_Developing_or_Dying_From_Cancer.asp?sitearea=
[11] Stewart BW, Kleihues P, eds. World Cancer Report. Lyon: IARC Press 2003.
[12] American Cancer Society. Cancer Statistics 2005 [PowerPoint]. Atlanta: American Cancer Society; 2005.
[13] MacKay J, Eriksen M. The Tobacco Atlas. Geneva: The World Health Organization; 2002.
[14] American Cancer Society. Cancer Prevention and Early Detection Facts and Figures 2007
Atlanta: American Cancer Society; 2007.
[15] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000 Jan 7;100(1):57-70.
[16] Dolan DNA Learning Center - Cold Spring Harbor Laboratory. Inside Cancer. [Multimedia Guide] 2004 [cited 2007 May 10]; Available from: www.insidecancer.org
[17] Cotran RS, Kumar V, Robbins SL. Robbins Pathologic Basis of Disease. 5th ed: W.B. Saunders Company 1994.
[18] National Cancer Institute. Milestone (1971): President Nixon declares war on cancer. 2007 [cited 2007 May 10]; Available from: http://dtp.nci.nih.gov/timeline/noflash/milestones/M4_Nixon.htm
[19] Niederhuber JE. The Nation's Investment in Cancer Research. Frederick, Maryland: National Cancer Institute; 2006 October.

[20] National Cancer Institute. The NCI Strategic Plan for Leading the Nation. Frederick, Maryland: National Cancer Institute; 2006 January.
[21] National Center for Health Statistics. Health, United States, 2006 With Chartbook on Trends in the Health of Americans. Hyattsville, MD; 2006.
[22] Carr DB, Loeser JD, Morris DB. Narrative, Pain and Suffering (Progress in Pain Research and Management). Seattle, WA: IASP Press 2005.
[23] Wang XS, Li TD, Yu SY, Gu WP, Xu GW. China: status of pain and palliative care. Journal of pain and symptom management. 2002 Aug;24 (2):177-9.
[24] U.S. Preventive Services Task Force. Guide to Clinical Preventive Services. 2nd ed. Alexandria, VA: International Medical Publishing 1996.
[25] Elmore JG, Armstrong K, Lehman CD, Fletcher SW. Screening for breast cancer. Jama. 2005 Mar 9;293(10):1245-56.
[26] Warner E, Plewes DB, Hill KA, Causer PA, Zubovits JT, Jong RA, et al. Surveillance of BRCA1 and BRCA2 mutation carriers with magnetic resonance imaging, ultrasound, mammography, and clinical breast examination. Jama. 2004 Sep 15;292(11):1317-25.
[27] Thomas DB, Gao DL, Ray RM, Wang WW, Allison CJ, Chen FL, et al. Randomized trial of breast self-examination in Shanghai: final results. Journal of the National Cancer Institute. 2002 Oct 2;94(19):1445-57.
[28] American Cancer Society. Cancer Facts and Figures 2006. Atlanta: American Cancer Society; 2006.
[29] Humphrey LL, Helfand M, Chan BK, Woolf SH. Breast cancer screening: a summary of the evidence for the U.S. Preventive Services Task Force. Annals of internal medicine. 2002 Sep 3;137(5 Part 1):347-60.
[30] Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. Vaccine. 2006 Aug 21;24 Suppl 3:S11-25.
[31] Nijhuis ER, Reesink-Peters N, Wisman GB, Nijman HW, van Zanden J, Volders H, et al. An overview of innovative techniques to improve cervical cancer screening. Cell Oncol. 2006;28(5-6):233-46.
[32] Burchell AN, Richardson H, Mahmud SM, Trottier H, Tellier PP, Hanley J, et al. Modeling the sexual transmissibility of human papillomavirus infection using stochastic computer simulation and empirical data from a cohort study of young women in Montreal, Canada. American journal of epidemiology. 2006 Mar 15;163(6):534-43.
[33] American Cancer Society. FDA Approves New Cervical Cancer Screening Test. 2003 March 31 [cited 2007 May 10]; Available from: http://www.cancer.org/docroot/NWS/content/NWS_1_1x_FDA_Approves_New_Cervical_Cancer_Screening_Test.asp
[34] Cohen J. Public health. High hopes and dilemmas for a cervical cancer vaccine. Science (New York, NY. 2005 Apr 29;308(5722):618-21.
[35] Alberts B. Molecular biology of the cell. 3rd ed. New York: Garland Pub. 1994.
[36] Renshaw AA. Measuring sensitivity in gynecologic cytology: a review. Cancer. 2002 Aug 25;96(4):210-7.
[37] Centers for Disease Control. Regulations for implementing the Clinical Laboratory Improvement Amendments of 1988: a summary. MMWR. 1992 (No. RR-2).
[38] Austin RM. Human papillomavirus reporting: minimizing patient and

laboratory risk. Archives of pathology & laboratory medicine. 2003 Aug;127 (8):973-7.
[39] Bogdanich W. Lax Laboratories: The Pap Test Misses Much Cervical Cancer Through Lab's Errors. The Wall Street Journal. 1987 November 2.
[40] Nanda K, McCrory DC, Myers ER, Bastian LA, Hasselblad V, Hickey JD, et al. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Annals of internal medicine. 2000 May 16;132(10):810-9.
[41] Fahey MT, Irwig L, Macaskill P. Meta-analysis of Pap test accuracy. American journal of epidemiology. 1995 Apr 1;141(7):680-9.
[42] Mitchell MF, Schottenfeld D, Tortolero-Luna G, Cantor SB, Richards-Kortum R. Colposcopy for the diagnosis of squamous intraepithelial lesions: a meta-analysis. Obstetrics and gynecology. 1998 Apr;91(4):626-31.
[43] Welch HG. Should I be Tested for Cancer? Berkely, CA: University of California Press 2004.
[44] Ferenczy A, Franco E. Cervical-cancer screening beyond the year 2000. The lancet oncology. 2001 Jan;2(1):27-32.
[45] Hutchinson ML. Assessing the costs and benefits of alternative rescreening strategies. Acta cytologica. 1996 Jan-Feb;40(1):4-8.
[46] Kurman RJ, Henson DE, Herbst AL, Noller KL, Schiffman MH. Interim guidelines for management of abnormal cervical cytology. The 1992 National Cancer Institute Workshop. Jama. 1994 Jun 15;271(23):1866-9.
[47] Jeronimo J, Morales O, Horna J, Pariona J, Manrique J, Rubinos J, et al. Visual inspection with acetic acid for cervical cancer screening outside of low-resource settings. Revista panamericana de salud publica = Pan American journal of public health. 2005 Jan;17(1):1-5.
[48] Birdsong GG. Automated screening of cervical cytology specimens. Human pathology. 1996 May;27(5):468-81.
[49] Groopman J. Contagion: Papilloma Virus. The New Yorker. 1999 September 13.
[50] Saslow D, Runowicz CD, Solomon D, Moscicki AB, Smith RA, Eyre HJ, et al. American Cancer Society guideline for the early detection of cervical neoplasia and cancer. CA: a cancer journal for clinicians. 2002 Nov-Dec;52 (6):342-62.
[51] Russell J, Crothers BA, Kaplan KJ, Zahn CM. Current cervical screening technology considerations: liquid-based cytology and automated screening. Clinical obstetrics and gynecology. 2005 Mar;48(1):108-19.
[52] Health Technologies Advisory Committee-Minnesota. New Technologies for Cervical Cancer Screening: Strategies To Lower The Rate Of Cervical Cancer. *Health Services/Technology Assessment Text (HSTAT)*: National Library of Medicine 1999.
[53] FDA. FDA Approves Expanded Use of HPV Test. 2003 March 31 [cited 2007 May 20]; Available from: http://www.fda.gov/bbs/topics/NEWS/2003/NEW00890.html
[54] Schiffman M, Herrero R, Hildesheim A, Sherman ME, Bratti M, Wacholder S, et al. HPV DNA testing in cervical cancer screening: results from women in a high-risk province of Costa Rica. Jama. 2000 Jan 5;283(1):87-93.
[55] Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Chapter 9: Clinical applications of HPV testing: A summary of meta-analyses.

Vaccine. 2006 Aug 21;24 Suppl 3:S78-89.
[56] Digene Corporation. Hybrid Capture® 2 High-Risk HPV DNA Test™. [cited 2007 May 20]; Product Insert]. Available from: http://www.digene.com/pdf/L2290-P.I,%20hc2%20HPV%20DNA%20Test%20US.pdf
[57] Germar MJ, Marialdi M. Visual inspection with acetic acid as a cervical cancer screening tool for developing countries. Review for the 12th Postgraduate Training Course in Reproductive Health/Chronic Disease 2003 [cited 2007 May 28]; Available from: http://www.gfmer.ch/Endo/Course2003/Visual_inspection_cervical_cancer.htm
[58] Coalition NCC. NCCC Section on the HPV Vaccine. 2007 [cited 2007 March 2]; Available from: http://www.nccc-online.org/hpv-vaccine.php
[59] Cohen J. High Hopes and Dilemmas for a Cervical Cancer Vaccine. Science (New York, NY. 2003;308:618-21.
[60] Kane MA, Sherris J, Coursaget P, Aguado T, Cutts F. Chapter 15: HPV vaccine use in the developing world. Vaccine. 2006 Aug 21;24 Suppl 3:S132-9.
[61] Sankaranarayanan R, Shastri SS, Basu P, Mahe C, Mandal R, Amin G, et al. The role of low-level magnification in visual inspection with acetic acid for the early detection of cervical neoplasia. Cancer detection and prevention. 2004;28(5):345-51.
[62] Pan American Health Organization. Visual Inspection of the Uterine Cervix with Acetic Acid (VIA): A Critical Review and Selected Articles. Washington, DC; 2003.
[63] Park SY, Follen M, Milbourne A, Rhodes H, Malpica A, MacKinnon M, et al. Automated Image Analysis of Digital Colposcopy for the Detection of Cervical Neoplasia. manuscript submitted for publication. 2007.
[64] Kasper D, Braunwald E, Fauci A, Longo D, Hauser S, Jameson JL, eds. Harrison's Principles of Internal Medicine. 16th ed. New York City: McGraw-Hill 2005.
[65] Hutchinson K, Wener M. National Health and Nutrition Examination Survey Lab Methods 2003-2004: Total Prostate-Specific Antigen (PSA): University of Washington Medical Center; 2007 May 4.
[66] Aureon Biosystems. LumiPhos Plus. [cited 2007 March 10]; Available from: http://www.aureonbio.com/ProductList.htm
[67] Lumigen. Lumigen PPD. 2006 [cited 2007 March 10]; Available from: http://www.lumigen.com/documents/LumigenPPD.shtml
[68] Hernandez J, Thompson IM. Prostate-specific antigen: a review of the validation of the most commonly used cancer biomarker. Cancer. 2004 Sep 1;101(5):894-904.
[69] Albertsen PC. Is screening for prostate cancer with prostate specific antigen an appropriate public health measure? Acta oncologica (Stockholm, Sweden). 2005;44(3):255-64.
[70] Gottlieb RH, Mooney C, Mushlin AI, Rubens DJ, Fultz PJ. The prostate: decreasing cost-effectiveness of biopsy with advancing age. Investigative radiology. 1996 Feb;31(2):84-90.
[71] American Cancer Society. How Is Prostate Cancer Diagnosed? 2006 July 26 [cited 2007 May 20]; Available from: http://www.cancer.org/docroot/CRI/content/CRI_2_4_3X_How_is_prostate_cancer_diagnosed_36.asp

[72] Lu-Yao GL, Yao SL. Population-based study of long-term survival in patients with clinically localised prostate cancer. Lancet. 1997 Mar 29;349 (9056):906-10.
[73] Neal DE, Leung HY, Powell PH, Hamdy FC, Donovan JL. Unanswered questions in screening for prostate cancer. Eur J Cancer. 2000 Jun;36 (10):1316-21.
[74] Bartsch G, Horninger W, Klocker H, Reissigl A, Oberaigner W, Schonitzer D, et al. Prostate cancer mortality after introduction of prostate-specific antigen mass screening in the Federal State of Tyrol, Austria. Urology. 2001 Sep;58(3):417-24.
[75] Horninger W, Berger A, Pelzer A, Klocker H, Oberaigner W, Schonitzer D, et al. Screening for prostate cancer: updated experience from the Tyrol study. The Canadian journal of urology. 2005 Feb;12 Suppl 1:7-13; discussion 92-3.
[76] Ilic D, O'Connor D, Green S, Wilt T. Screening for prostate cancer: a Cochrane systematic review. Cancer Causes Control. 2007 Apr;18(3):279-85.
[77] Amling CL. Prostate-specific antigen and detection of prostate cancer: What have we learned and what should we recommend for screening? Current treatment options in oncology. 2006 Sep;7(5):337-45.
[78] Hennekens CH, Buring JE. Epidemiology In Medicine. Philadelphia: Lippincott Williams & Wilkins 1987.
[79] Welch HG, Schwartz LM, Woloshin S. Are increasing 5-year survival rates evidence of success against cancer? Jama. 2000 Jun 14;283(22):2975-8.
[80] Reynolds EA, Moller KA. A review and an update on the screening of epithelial ovarian cancer. Current problems in cancer. 2006 Sep-Oct;30(5):203-32.
[81] Rosenthal AN, Menon U, Jacobs IJ. Screening for ovarian cancer. Clinical obstetrics and gynecology. 2006 Sep;49(3):433-47.
[82] BC Cancer Agency. Years of Life Lost. 2005 July 25 [cited 2007 May 22]; Available from: http://www.bccancer.bc.ca/HPI/CancerStatistics/FF/LifeLost.htm
[83] Petricoin EF, Ardekani AM, Hitt BA, Levine PJ, Fusaro VA, Steinberg SM, et al. Use of proteomic patterns in serum to identify ovarian cancer. Lancet. 2002 Feb 16;359(9306):572-7.
[84] Urban N. Specific keynote: ovarian cancer risk assessment and the potential for early detection. Gynecologic oncology. 2003 Jan;88(1 Pt 2):S75-9; discussion S80-3.
[85] Nahhas WA. Ovarian cancer. Current outlook on this deadly disease. Postgraduate medicine. 1997 Sep;102(3):112-20.
[86] Zurawski VR, Jr., Sjovall K, Schoenfeld DA, Broderick SF, Hall P, Bast RC, Jr., et al. Prospective evaluation of serum CA 125 levels in a normal population, phase I: the specificities of single and serial determinations in testing for ovarian cancer. Gynecologic oncology. 1990 Mar;36(3):299-305.
[87] Gladstone CQ. Screening for ovarian cancer. In: Canadian Task Force on Periodic Health Examination, ed. *Canadian Guide to Clinical Preventive Health Care.* Ottawa: Health Canada 1994:870-81.
[88] Menon U, Jacobs IJ. Ovarian cancer screening in the general population. Current opinion in obstetrics & gynecology. 2001 Feb;13(1):61-4.
[89] van Nagell JR, Jr., DePriest PD, Reedy MB, Gallion HH, Ueland FR,

Pavlik EJ, et al. The efficacy of transvaginal sonographic screening in asymptomatic women at risk for ovarian cancer. Gynecologic oncology. 2000 Jun;77 (3):350-6.
[90] Lok IH, Sahota DS, Rogers MS, Yuen PM. Complications of laparoscopic surgery for benign ovarian cysts. The Journal of the American Association of Gynecologic Laparoscopists. 2000 Nov;7(4):529-34.
[91] Hensley ML, Spriggs DR. Cancer screening: how good is good enough? J Clin Oncol. 2004 Oct 15;22(20):4037-9.
[92] Johnson D, Sandmire D, Klein D. Ovarian Cancer. *Medical Tests That Can Save Your Life: 21 Tests Your Doctor Won't Order Unless You Know to Ask*. Emmaus, PA Rodale 2004.
[93] Fees Involved in Infertility Treatments. 2005 [cited 2007 June 1]; Available from: http://www.sharedjourney.com/articles/eggfees.html
[94] Jacobs IJ, Skates S, Davies AP, Woolas RP, Jeyerajah A, Weidemann P, et al. Risk of diagnosis of ovarian cancer after raised serum CA 125 concentration: a prospective cohort study. BMJ (Clinical research ed. 1996 Nov 30;313(7069):1355-8.
[95] Jacobs IJ, Skates SJ, MacDonald N, Menon U, Rosenthal AN, Davies AP, et al. Screening for ovarian cancer: a pilot randomised controlled trial. Lancet. 1999 Apr 10;353(9160):1207-10.
[96] UK Collaborative Trial of Ovarian Cancer Screening. 2006 July 11 [cited 2007 May 20]; Available from: http://www.ukctocs.org.uk/index.html
[97] Buys SS, Partridge E, Greene MH, Prorok PC, Reding D, Riley TL, et al. Ovarian cancer screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial: findings from the initial screen of a randomized trial. American journal of obstetrics and gynecology. 2005 Nov;193(5):1630-9.
[98] FDA. Protein Patterns May Identify Ovarian Cancer. 2002 February 7 [cited 2007 May 20]; Available from: http://www.fda.gov/bbs/topics/NEWS/2002/NEW00797.html
[99] Pollack A. New Cancer Test Stirs Hope and Concern. The New York Times. 2004 February 3.
[100] Underwood A. Testing: Ovarian Cancer. Newsweek. 2002 February 18:12.
[101] Baggerly KA, Morris JS, Coombes KR. Reproducibility of SELDI-TOF protein patterns in serum: comparing datasets from different experiments. Bioinformatics (Oxford, England). 2004 Mar 22;20(5):777-85.