

Onco-Immunology: Breaking the First Stage of Anergy

Jesse Stoff, MD, FAAFP



Anergy is an immunological term that describes a lack of reaction by the body's defense mechanisms to foreign substances, and results in the induction of peripheral lymphocyte tolerance. Testing for and breaking anergy is the first step towards immune reconstitution. Immune reconstitution is the evolving clinical science of restoring immune competence. Such interventions must be ascertained for each individual, as the anergic defect(s) can vary widely, and broadly fall under the umbrella of "immunotherapy." It can include cancer vaccines, cytokine therapy, nutraceutical supplementation, lifestyle changes, the administration of monoclonal antibodies to modulate immune checkpoints, and others.

Owing to the wide antigenic variability of cancer, individualized cancer therapy is a central goal of onco-immunologists. Immunotherapy is a rational means to this end because, the immune system can recognize a virtually limitless number of antigens secondary to the activity of the antigen presenting cells and the biology of the genetic recombination of the B and T lymphocytes.

The immune system is exquisitely structured to distinguish self from non-self, and is able to vigorously attack non-self, cancer cells. The immuno-editing theory suggests that the immune system is able to recognize and eradicate the cancer cells that everyone seems to produce everyday, and subclinical tumors. However, if the immune system is compromised then, at some point, equilibrium is reached and the tumor remains in situ, in a state of balance with but a partially efficacious response. Unfortunately, many tumors then escape from this equilibrium state, and cancer becomes clinically apparent.

The goal of the onco-immunologist is to understand the mechanisms of anergy by which cancer is able to escape the immune system and to therapeutically intervene at critical points to promote anti-tumor immune responses through a process of immune reconstitution. When the immune system fails to recognize a specific foreign invader, it is said to be anergic. Anergy is an immunological term that describes a lack of reaction by the body's defense mechanisms to foreign substances, and results in the induction of peripheral lymphocyte tolerance. Molecules and cell surfaces that are identified as foreign are referred to as antigens and have the ability to elicit an immune re-sponse from a healthy immune

system. Testing for and breaking anergy is the first step towards immune reconstitution. Immune reconstitution is the evolving clinical science of restoring immune competence. Such interventions must be ascertained for each individual, as the anergic defect(s) can vary widely, and broadly fall under the umbrella of "immunotherapy." It can include cancer vaccines, cytokine therapy, nutraceutical supplementation, life-style changes, the administration of monoclonal antibodies to modulate immune checkpoints, and others.

Introduction

All diseases, either acute and severe or chronic, have, to some degree, an element of immune dysfunction that is central to the disease process.¹ The immune system is one of our primary and most critical systems, and helps to regulate our internal disease-fighting environment. It exerts its control by virtue of a multitude of circulating components, some of which include cytokines that are capable of acting at sites far removed from their points of origin. Its complexity rivals that of the nervous system, and in fact the similarities between the two are quite real. Cells of the immune system and the nervous system have many hormone receptor sites in common.² It is no accident of nature that the thymus gland, the bone marrow, and the lymph nodes--all major centers of immune activity--are bundled in ropes of nerves. The brain is known to transmit both electrical and chemical signals along nerves to stimulate, amplify and modify the immune responses.³ As the signals stream out from the brain, they often pass warnings from the immune centers flying in the op-

posite direction. The immune system is not merely a tool that is manipulated by the brain, but rather it is a sensory organ as well. It transmits chemical messages about bacteria, fungi, viruses, bits of dead tissue, and cancer cells. The wonder of it all is that such organization is possible, with the use of only a few distinct cell types whose members are widely scattered throughout the body.

The immune system can be used as a therapeutic modality, especially as an anticancer agent, due to its flexibility and exquisite specificity. The reliance of this therapy on naturally occurring biological molecules to augment and guide the immune response means that of all the scientifically validated methods for treating cancer, immunotherapy may be the most natural and familiar to the patient. The immune system has the capability to spontaneously change its response as the cancer mutates; therein lies the promise of Onco-immunology as a treatment option. It is also the one modality that benefits most from the true integration of the mind-body continuum to achieve its remarkable results.⁴

It is well established that the early detection of cancer is extremely important in the management and treatment of the disease. The reactivation of the immune system is our best defense for the early detection and response against cancer cells.⁵ By taking advantage of the tremendous recognition capacity of the immune system, immunologists hope to develop even more sensitive and effective cancer diagnostic and therapeutic tools.

Recognizing the Clinical Features

The immune system is not a pathway, it's a cascade of information that gets passed along, focused and amplified to destroy the targeted cancer cells. Some of the key pathways are depicted in Figure 1. Blood tests are clinically available to identify the competence or level of dysfunction of each step of the immune cascade.

This article will focus on the first interactions of the immune system as depicted in Figure 2.

The immune system operates according to three directives. The first is to **Recognize** that which is foreign and sound the alarm, in an attempt to thwart the invader and is the subject of this article. The second directive is to **Respond** to the alarm with enough of a counter attack to effectively neutralize the invader quickly. The third directive is to **Remember** what happened so that if the same situation were to arise again an effective response could be generated faster.

These immunologic directives are accomplished with but a handful of cells, cytokines and chemokines. One cell type is the macrophage and acts in a similar manner to the videogame hero Pacman, crawling around inside of us looking for something foreign to chomp down on. In doing so, it sends out a biochemical alarm as it recognizes something alien.⁵

Macrophages lead their own separate lives, yet under our direction; in some odd way, to protect us from danger. Perhaps the most fascinating aspect of macrophage activity, or the activity of any of the immune system cells, is their ability to distinguish "self" from "non-self." Through the integrity of the immune system we remain separate from our environment. This inner image of "self-ness" or uniqueness somehow carries through to each defensive cell as it works to eliminate foreign cells such as cancer from within, or virus particles as they penetrate in from the outside world. The mechanisms by which this image of "self" is maintained is still largely unknown.

We do, however, know that this mechanism exists, and more importantly, that it is subject to change. Macrophages, for example, do not merely mope about hoping to bump against a bacterium or other source of food. They migrate from distant corners of the body, zero in on targets that they know are alien, and proceed to destroy them. Some of this directed migration is orchestrated by cytokines and chemokines, some of which are released by dendritic cells. Dendritic cells are located

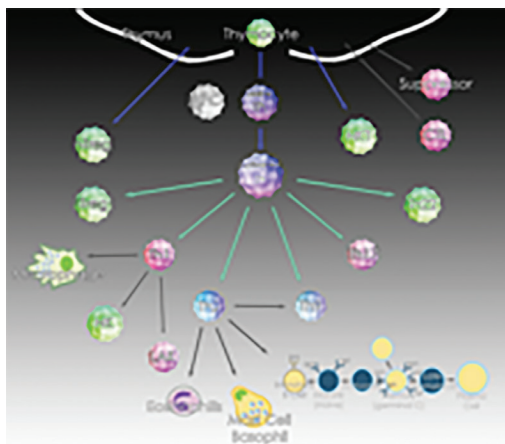


Figure 1
Key Pathways

within the structures of organs including the lungs, stomach, intestines and the skin. Dendritic cells located within the skin are referred to as Langerhans cells.⁷ Some of these cells have evolved from Monocytes and morph into Dendritic Cells as their services are needed in specific areas of the body to "investigate" some change in the local environment. Their main function is to identify foreign material, break it down and "present" it on their surface to immature T cells and other immunologic cells.

Along with the antigen, naïve (unstimulated, immature) T cells require co-stimulatory signals, along with the help of dendritic cells, to become fully activated. Together, they form a sort of immunologic synapse in which targeting information is transferred and an immune response cascade initiated.⁸ When all is working normally, we are protected from a wide variety of threats both from within our body and from the external environment. To maintain balance, there are normal pathways within the array of immune responses that allow for a sort of selective immunological "blindness," called tolerance.

Tolerance refers to specific immunological non-reactivity to an antigen. This comes in handy during pregnancy, as well as protecting us from autoimmune diseases. Tolerance is a natural phenomenon, and can be built into our own cells as well as acquired from our environment. For example, tolerance can be obtained by exposure to certain foods or animal particles. Tolerance develops through three pathways: central tolerance, peripheral tolerance and acquired tolerance. An aberrant, pathological form of tolerance is called anergy.

Technically speaking, anergy is defined as a state in which helper T lymphocytes are

available but incapable of producing the cytokine IL-2 and expanding their population in response to optimal antigenic stimulation. Anergy occurs when the T cell receptor (TCR) is engaged by antigen but in the absence of co-stimulation by dendritic cells or IL-2.⁹ Cytokines are small cell-signaling molecules that can be classified as proteins, peptides, or glycoproteins. The term cytokine is generally used to refer to immune stimulating molecules such as interleukins and interferons.¹⁰ IL-2 is a growth factor for all subpopulations of T-lymphocytes and induces cell cycle progression in resting cells and thus allowing for clonal expansion of activated T-lymphocytes. It has anti-tumor activity by its induction of the secretion of tumoricidal cytokines (such as TNF) and stimulates the expansion of LAK cells.¹¹

Anergy is of critical importance because it directly correlates to the stage of cancer: over 90% of patients with Stage 4 disease are found to be anergic.¹² The immune system cannot fight something that it doesn't know is there, and that's exactly what arises with anergy. Anergy manifests when there is a failure of signal transmission at ANY point in the immune response cascade.

Before we can break anergy, and wake up the immune system, we must make sure that we are dealing with true anergy as opposed to another pathology. Testing for nutritional deficiencies and the presence of immuno-suppressive toxins may be important depending upon the patient's history and chronology of their disease process.

Of historical interest, some years ago the Mérieux Company manufactured a skin test called the "Multitest Mérieux" or "CMI Multitest" system (Istituto Merieux Italia, Rome, Italy). It is used as a general test for the level of the cellular immune response. It is an intradermal test of skin reactivity (similar to allergy tests) in which a control (glycerol) is used with seven common antigens of bacterial or fungal origin (tetanus toxoid, tuberculin, diphtheria, streptococcus, candida, trichophyton, and proteus).

In this test, reactions are categorized according to the number of antigens provoking a response, and the summed magnitude of the skin response to all seven antigens. Based on the chart and information supplied with the simple skin test, anergy can be quickly assessed and quantified.¹³ Unfortunately, this test cannot tell us where in the recognition/response chain of events the problem lies. However, specialized blood tests are now able to do that. Once this state of unresponsiveness has been confirmed, the next step in breaking

anergy is with an immunotherapy protocol specific to the area(s) of immune response dysfunction. The next section will focus on the first step of initiating an immune cascade, recognition.

Antigen Presenting Cell Anergy

Breaking anergy will, at least briefly, restore the immune system's ability to identify a foreign body and send out a flood of cytokines to alert the rest of the immune defenders, and thus initiate a responsive cascade. Recognition depends upon the structural and functional integrity of the Antigen Presenting Cells (APCs). A blood test called a Phagocytic Index directly measures the activity of the macrophages and is taken as an indirect measure of the functional activity of the Dendritic Cells.¹⁴ It is calculated as the average number of bacteria ingested by each macrophage, in an individual's blood, after a mixture of the blood serum, bacteria, and phagocytes have been incubated per the protocol's period of time. Depending upon how suppressed the immune system is (and for what reasons) the elevation of the Phagocytic Index, after stimulation, can last for 1 to several weeks.

To counter this level of anergy, there are several reliable options for stimulating APC's in a relatively short period of time including:

- B-1,3-Glucan
- Dendritic Cell Vaccine
- BCG
- PNEUMOVAX® 23
- Gc-MAF

Of these, injectable B-1,3-Glucan is not currently available in the United States. Dendritic Cell Vaccines not only stimulate APCs, but have been shown to be effective against many forms of cancer. However, the only form currently available in the United States is a variation that is called Provenge® (Sipuleucel-T), and it is used for advanced Prostate cancer.

Bacillus Calmette-Guérin (historically known as Vaccin Bilié de Calmette et Guérin currently referred to as Ba-cille de Calmette et Guérin or BCG) is a vaccine that is commonly given in many countries around the world as a modicum of protection against tuberculosis. It is prepared from an attenuated strain of the live bovine tuberculosis bacillus, *Mycobacterium bovis*. Through a special process of sub-culturing, a less virulent strain has been created to use for this purpose. When injected intra-dermally it will form a local infection that can smolder for months, all the while stimulating APC activity systemically. BCG can be infused into the bladder to generate

a local immune/inflammatory response that can stop some early, superficial bladder cancers better than the chemotherapy that it was tested against, according to the product insert.¹⁶

PNEUMOVAX® 23 vaccine contains polysaccharides that can stimulate APC's locally and T & B cells systemically.

Macrophages can also be stirred into action by a glycoprotein called Group-specific Complex-Macrophage Activating Factor, Gc-MAF, that is normally produced by the body. Cancerous cells secrete several abnormal enzymes into the bloodstream such as EctoNox 2 and alpha-N-acetylgalactosaminidase (NaGalase). NaGalase causes the de-glycosylation of serum vitamin D3-binding protein (known as Gc protein), that is a precursor for the production of macrophage activating factor (MAF). Subsequently, this blocks Gc-MAF's production and activity. Therefore, macrophages of cancer patients having deglycosylated Gc protein cannot be activated, leading to immunosuppression of these critical antigen-presenting cells. This suppressive effect can be temporarily overcome by administering Gc-MAF. The Gc-MAF seems to work on several cell types to help initiate an immune response. Furthermore, studies have demonstrated the ability of Gc-MAF to decrease angiogenesis and stimulate the production of several chemokines.¹⁷

Chemokines play a major role within the immune system and act as a molecular "magnet" to guide the migration of cells. Specific cells are attracted by specific chemokines and follow the signal of increasing chemokine concentration towards the source of its release. Their classification is based on their structural characteristics, not just their ability to attract cells.¹⁸

Interleukin 2 (IL-2) is a (cytokine) type of immune communication molecule that

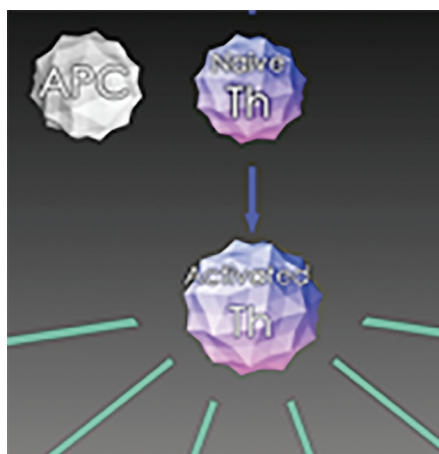
regulates the growth and differentiation of T cells. It is secreted by antigen-stimulated T cells and pushes the development of immature CD4 cells into the Th1 direction. This is important because it is the Th1 cells that largely co-ordinate the immune counter-attack against cancer cells.

How I Treat APC Anergy

To help re-engage the macrophages and break anergy, at the level of recognition, I first prepare a vial with equal parts Gc-MAF, Pneumovax® and IL-2.^{19,20,21} With the cocktail thus prepared I first apply Aldara® to the injection site. Aldara® (Imiquimod), when applied to the skin, can lead to the activation of Langerhans cells (skin Dendritic Cells), which subsequently migrate to local lymph nodes to activate the adaptive immune system.²² Presensitizing the area with Aldara® and then injecting the APC stimulant mix seems to give a faster, better response. Then I inject 0.01 cc intradermally, like an allergy test injection (Figure 3), every other day until I see a local reaction, similar to a bee sting.²³ Then, I give a full dose of Pneumovax® (0.5 cc) intramuscularly (IM). It's important to have, and know how to use, the usual drugs needed to treat anaphylaxis, as it is a theoretical possibility.²⁴

I hypothesize that, among other things, this cocktail causes the release of the chemokine, MCP-1. When released, this chemokine then attracts macrophages into the area which, along with skin dendritic cells, processes the Pneumovax® LPS antigens and begins an immune cascade with a cytokine release that results in the systemic activation of antigen presenting cells. Furthermore, monocytes from capillaries damaged by the injections are stimulated by the GcMAF and exert a critical influence on the induction of protective T-helper (Th) responses.^{25,26}

Figure 2
First Interactions of the Immune System



Nutraceuticals

Over decades of research some nutraceuticals have proven helpful in supporting immune, and specifically Th1, function. In advanced states of disease, they can play a useful part, but rarely a definitive role, when they act as immune response modifiers to help nurture the healing path. Immune response modifiers are characterized as having a directional effect, which is often dose dependent and biphasic, on the immune system without the benefit of responding differentially to feedback loops. Some of the agents that support/stimulate the Th1 cells or suppress the Th2 cells and thus shift the Th1/Th2 balance in a Th1 direction are (Chart 1):

Chart 1
Agents that Support/Stimulate Th1 Cells or Suppress Th2 Cells

	Th1	Th2
Melatonin promotes ²⁷	X	
Refined sugar suppresses ²⁸	X	
Zinc deficiency increases ²⁹	X	
Quercetin promotes ³⁰	X	
Lactobacillus supports ³¹	X	
EPA/DHA Fish Oil low dose supports ³²	X	
Vitamin E low dose supports ³³	X	
Arabinogalactan stimulates ³⁴	X	
Vitamin C promotes ³⁵	X	
Cipro and some other antibiotics suppress ³⁶	X	
Thyroid hormones support ^{37, 38}	X	
Garlic stimulates ³⁹	X	
Moderate exercise increases ⁴⁰	X	
Glutathione stimulates ⁴¹	X	
Glucans from therapeutic yeasts and fungi stimulate ⁴²	X	
Magnesium deficiency increases ⁴³		X
Over cooked/processed omega-6 vegetable oils supports ⁴⁴		X
Stress increases cortisol release and stimulates ⁴⁵		X
Antigen Immunotherapy suppresses ⁴⁶		X
Mercury and lead promote ^{47, 48}		X
Alcohol stimulates ⁴⁹		X
Cigarette tars and nicotine stimulate ⁵⁰		X

Only one nutraceutical has proven effective as a true immune modulator, in that it activates both sides of the immune cascade receptors and thus is not an immune stimulant, which has a one sided effect.⁵¹ That nutrient, AiE10, I find to be helpful in that it can speed up the reconstitution of immune function.

Discussion

Usually, this protocol is effective and will result in a significant immunological response. There will be pain, swelling, and heat (inflammation) around the IM injection site, and at times produces a fever and other "flu-like" symptoms. It is important NOT to treat these symptoms with NSAIDs, because these reactions are a reflection of the immune response that we want. If the fever goes above 102°F, which is extremely rare, then Tylenol® may be judiciously used. Immunologically, I then can track and measure the breaking of anergy with the Phagocytic Index. I have seen cases where just breaking this step of anergy was enough to regenerate an immune response capable of putting an early cancer into remission. Stimulated macrophages produce another cytokine called IL-12, which is also involved in the differentiation of naive T cells into Th1 cells.⁵² The stimulated Th1 cells then produce a series of cytokines

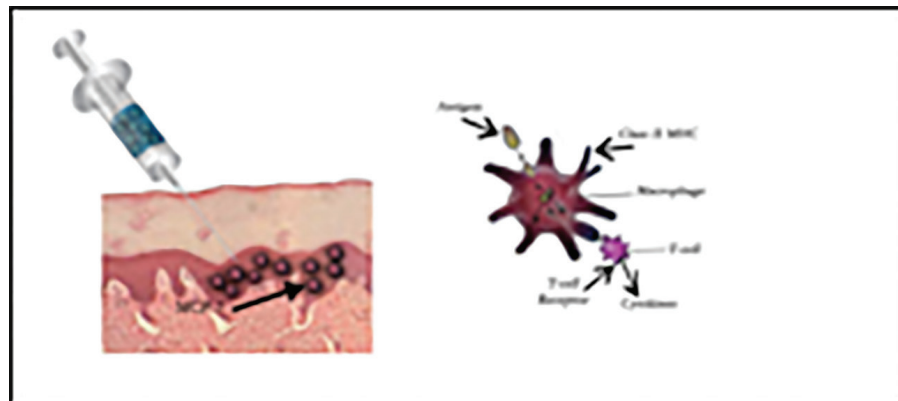
which, amongst other things, stimulate the natural killer (NK) cells, that are tasked with destroying virally infect-cells and cancer cells. The increased processing of antigens by dendritic cells and macrophages is a window of opportunity for initiating the next critical steps of the immuno-therapy protocol for breaking anergy.^{53,54,55} These steps include unmasking cancer cells, Th1 stimulation, support and balance, NK stimulation and support, psychoneuroimmunology, detoxification of the immune system.

The field of Onco-immunology is quietly exploding. Progress is being made on many fronts simultaneously. Recent advances in Immunology have enabled scientist to generate mono-clonal antibodies for therapeutic use. They attach to targeted cells or proteins which are then destroyed by macrophages. Some of the antibodies available to help fight cancer include Gemtuzumab⁵⁶ for relapsed myeloid Leukemia, Trastuzumab⁵⁷ for HER2/neu positive breast cancers and Rituximab⁵⁸ for non-Hodgkin's lymphoma. These therapies largely work independently from the patient's acquired immune system, and so the long term benefit has been limited.

Other strategies try to generically stimulate immune function without regard to the status of the patient's immune system. One such therapy uses Dendritic cell-derived exo-somes. Exosomes⁵⁹ are cell derived vesicles that can contain enzymes, RNA and other nucleoproteins (like transfer factor) that can be transferred from one cell to another. Exosomes harbor a discrete set of proteins, bear functional MHC class I and II molecules that can be loaded with synthetic peptides of choice, and can be used to simulate APC-like activity. Another strategy works a bit further down the path of the immune cascade but again without benefit of assessment to the integrity of the patient's immune system. This strategy uses chimeric antigen receptors (CARs)⁶⁰, which usually combine the antigen binding site of a monoclonal antibody with the signal activating machinery of a T cell.

This is analogous to the later part of antigen recognition. By freeing antigen recognition from major histocompatibility complex restriction and thus breaking one of the barriers to the initiation of the immune cascade, this promising therapy can jump-

Figure 3
Dendritic Cells. Compared to other vaccination sites, the skin has relatively high numbers of Dendritic cells which, along with the migration of Macrophages into the injected area, can elicit a potent systemic cellular immune response.



start an immune response if the APC's are at fault. If the lack of an effective immune response is from damage further down the cascade then the response will be very limited. However, such engineered autologous T-cells that are grown into large quantities ex vivo and then re-infused have produced remarkable responses in late stage refractory CLL patients and now the concept is being applied to other B-cell malignancies with promising early results.

Conclusion

Much research has been done developing antigen vaccines for cancer. Historically, they have not performed very well. In the last millennium, as a Family Practice resident, I was involved with the development of a potential vaccine for colon cancer; it didn't work. It and others like it don't work, because generally speaking, it is not a lack of cancer antigens present to elicit an immune response, but rather the recognition or response is impeded due to anergy. Appreciating and then breaking anergy is the first critical step for initiating a successful immuno-therapeutic protocol. There are several key immunological reactions that may fail thus resulting in anergy. Each must be identified and overcome in order to restore immune balance.

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Dr. Stoff is an internationally renowned physician, with extensive credentials in immunology, naturopathy, homeopathy, acupuncture, and holistic medicine. He is a licensed Medical Doctor, a Certified Naturopathic Physician, a Certified Acupuncturist, and a licensed Homeopathic Physician. A graduate of New York Medical College, he pursued extensive post-doctoral training including a fellowship at the Royal London Homeopathic Hospital in London, England. He has authored/co-authored dozens of articles and eight books including co-authoring the bestsellers *Chronic Fatigue Syndrome: The Hidden Epidemic* and *The Prostate Miracle*. He has also served as a member of the Clinical Nutrition Board of Cancer Treatment Centers of America, Inc. As Medical Director of the Stoff Institute for Medical Research, he consults with physicians and medical groups both domestically and abroad on the subjects of immune system disorders and immune reconstitution. As a result of his research he has developed several new molecular complexes, one of which is now being patented as a true anti-biotic replacement.

Dr. Stoff sub-specializes in Onco-immunology, which he lectures on at national and international medical conventions, and has been doing IPT intermittently since the mid 90's.

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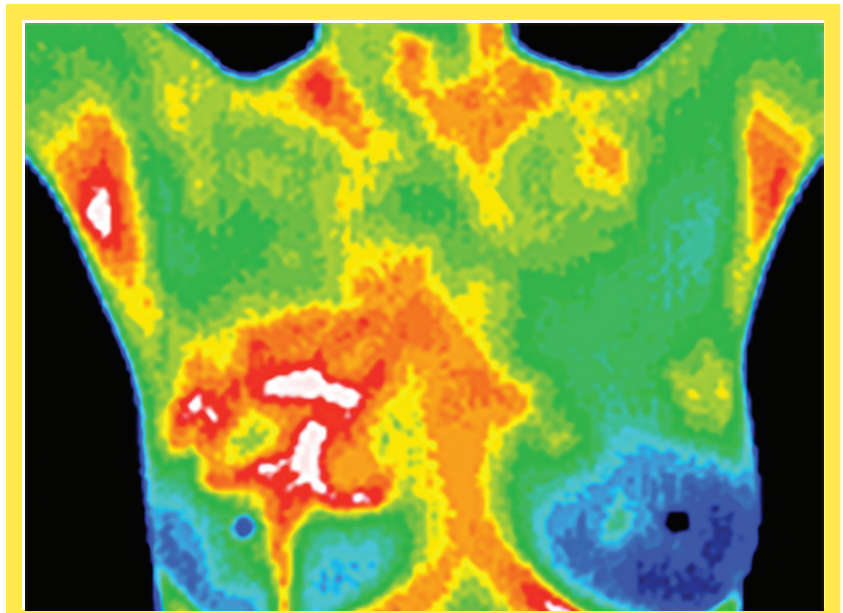
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