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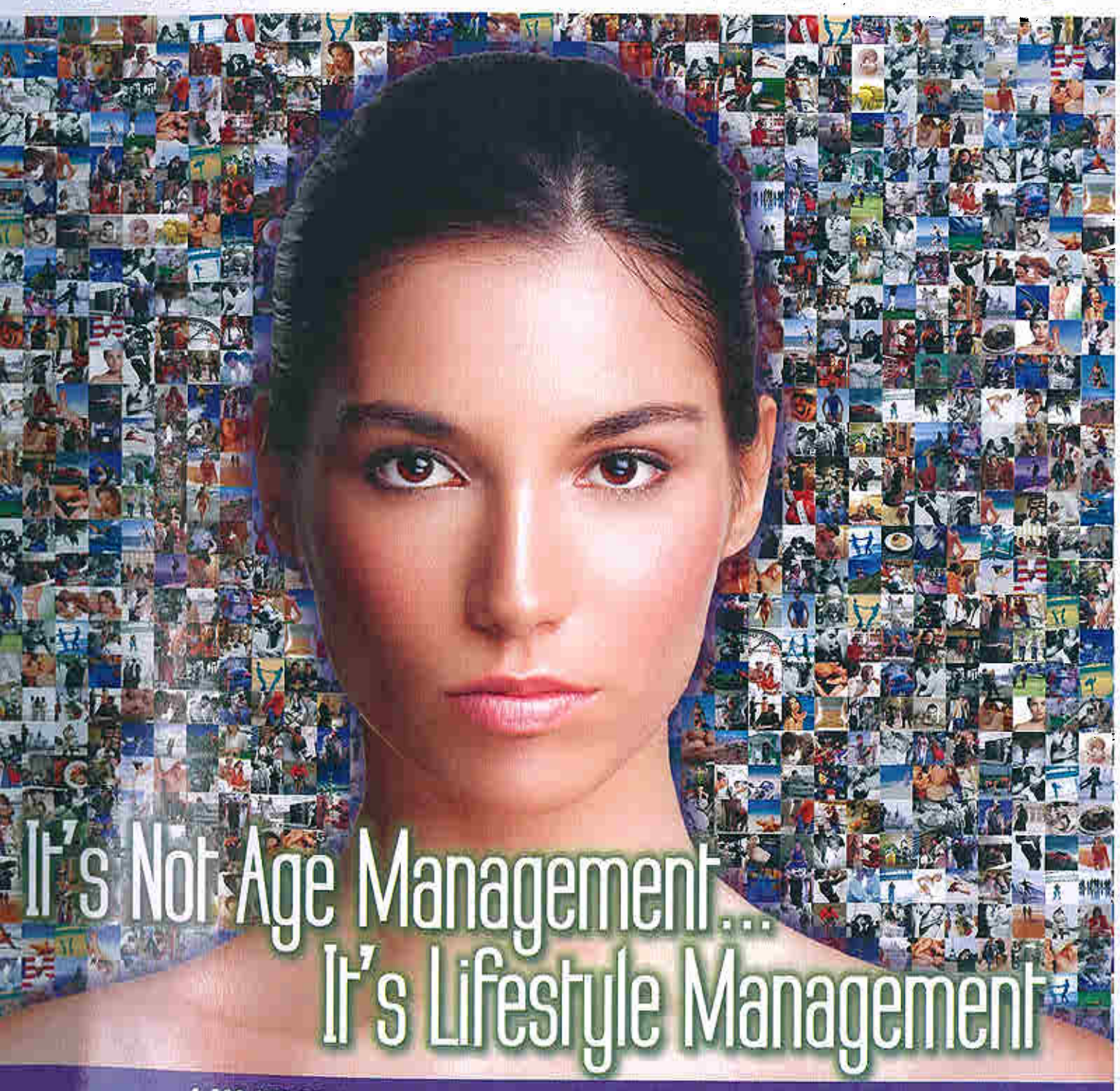

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Telomere Measurements in Clinical Medicine- How, What, When, and Why!

By Dave Woynarowski, MD

Why has telomere measurement become so important?

The medical and public consciousness about the importance of telomeres and telomere length has exploded as the result of the 2009 Nobel Prize Award for the discovery of telomerase, the enzyme that elongates telomeres, slowing the aging process. Since then, both clinicians and the lay public have been inundated with studies correlating telomere length to diet, supplements, sleep, stress and all manner of lifestyle habits. This is on top of an even larger data base of more than 17,000 scientific publications correlating telomere length with diseases we commonly associated with aging such as cancer, heart disease (both vascular and myopathyic), Alzheimer's, arthritis (both degenerative and autoimmune) diabetes, infertility and many other age-related diseases.

As a result of the known loss of telomere length from the ends of the chromosomes with each cell division and the initial suggestion that this loss was limiting to the life span of the cell (the Hayflick Limit) the concept that telomere loss may actually cause aging arose.

The current understanding is that telomere loss will eventually limit the lifespan of cells tissues and organisms and is correlated with the aging process because it correlates well with the organism's ability to produce new healthy cells to replace sick dead or dying cells. As the organism ages and the telomeres shorten the regenerative and repair capacity is limited by the numbers of cells that have long enough telomeres to function in healthy fashion and reproduce healthy offspring in whatever tissue is being studied.

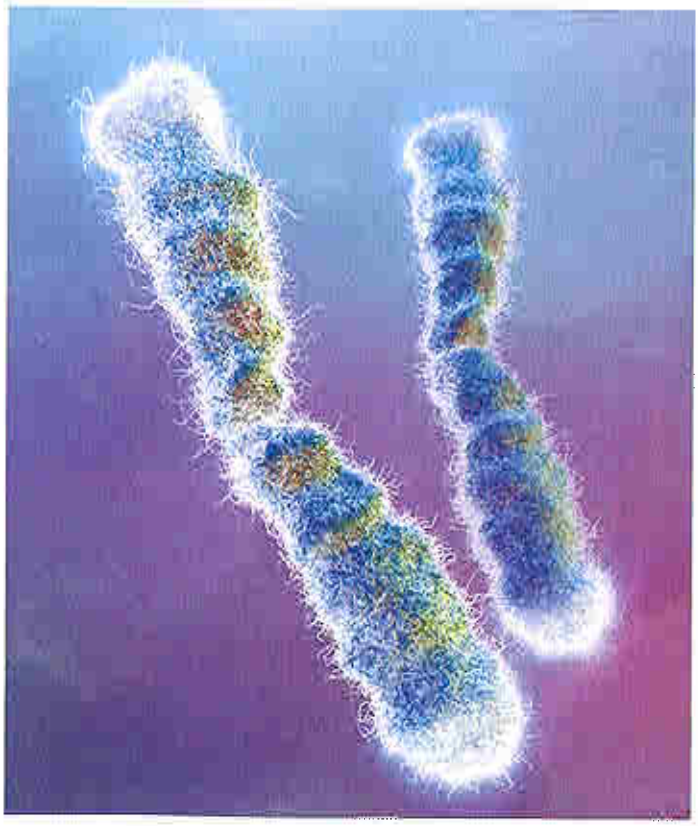
As a result many models have been applied to try to understand just how significant telomere length is as a "key driver" of human aging versus a mere biomarker.

What we know for sure

- Telomere length and loss sets into motion at least two possible regulatory "checkpoint" outcomes that limit cell life and cell replication: senescence and apoptosis. We know that in at least certain tissues these changes lead to what is now called the "Senescent Activated Phenotype" which involves the inflammatory activation of the immune system and collateral damage to surrounding cell masses that may be neither damaged nor sick. In a very real sense, aging may accelerate aging.

- Replicative demand and stress (most commonly oxidative or nitrosative) are the two main factors in telomere loss. Importantly, both are subject to clinical interventions by the doctor and patient.

- We know that the expression of telomerase is responsible for the genomic preservation of germ line cells, part of the resistance of stem cells to oxidative damage and their enhanced longevity and that the bulk of cells in our body referred to as "somatic cells" have little or no telomerase



expression due to telomerase repression that is not present in the aforementioned cell lines to the same degree.

- We know that longer telomere lengths appear to be protective against cancer but that in approximately 85% of human cancers high levels of telomerase are required for survival of the cancer.

- We know that in cell cultures containing healthy cells with a normal genome the normal limits of replication can be extended to what has been termed "functional immortality" with the introduction and expression of the telomerase enzyme and the obligate subsequent extension of telomere length. (Bodnar et al.)

- We know that telomerase expression may be the only realistic and viable method of mammalian life extension as was demonstrated in mice (Vera et al.).
- And finally we know that overall average telomere length does not correlate nearly as well with cellular dysfunction and aging but the presence of increased numbers of critically short telomeres does.

- The presence and degree of "critically short telomeres" as opposed to average or mean telomere length is increasingly recognized as the driver of aging and cellular dysfunction. It is also the basis for "biologic age" determination. Biologic age is how old the cell tissue or organism acts and functions as opposed to how many years it has been alive.

This information gives us a useful working definition of aging and a useful set of parameters to follow based on telomere measurements. All that remains now is to see if all of these things hold true in larger and larger human databases!

Part of the difficulty is due to the fact that initial studies involved large groups of participants and utilized the same technologies that were developed in basic science during the study of single celled organisms. This set a precedent of specific testing modalities that may actually not be all that useful in individual human testing.

Subsequently successful attempts were made to transition what was once only a basic science tool into potential use for clinical studies and eventually for the individual clinician with the interested patient. It has only been recently however that the later technology has been refined.

As is often the case with cutting edge technologies, the physician who practices Anti-Aging Medicine or some form of Age Management has become the pioneer in this and is asked by the ever more educated and discerning patient to answer the question of biologic age by utilizing telomere testing.

In a very real sense those of us who measure our patients' telomeres will be the ones to answer this question posed above: Do all of these things hold true in larger and larger human databases?

It is imperative therefore that you understand some basic concepts:

1) The most commonly measured cell line for assessment of telomere length are the Peripheral White Blood Cell (PBMC's) lines including granulocytes and/or lymphocytes. While different tissues age differently and have distinctively different telomere lengths and loss rates there is a general and useful correlation between peripheral white blood cell measurements and the general health status of the individual and their actual functional or biologic age. For example there is a well established correlation between outcomes in heart disease and WBC telomere lengths (Benetos et al.)

2) White blood cell lines are changeable with the patient's health the patient should be in relatively "stable health" both mentally and physically since either parameter can affect numbers of cells and their telomere length. Bottom line: Do not test telomere length when your patient is (atypically) stressed or sick.

3) The actual loss or in fewer cases gain of telomere length can be best assessed by time intervals of one or more years depending on the type of telomere testing you decide to use. I cannot stress enough the need to commit to more than one telomere test at some defined time interval (such as annually) to get meaningful information.

4) Since the presence and more specifically the number or percentage of short telomeres is correlated with activating the DNA damage response that leads to cell cycle arrest or apoptosis it is the target of research focusing on differentiating biologic age from chronologic age.

5) Typical telomere attrition in PBMC's is estimated to range between 50 to 200 base pairs (bps) per year.

6) Many test technologies have reproducibility issues and inaccuracies that exceed the actual number of base pairs lost by 2 and 3 fold, making interpretation more difficult and less meaningful. In these cases it would be better to wait several years to repeat the test.

7) At this time only there is one proprietary HT Q-FISH technology which allows for the determination of biologic age.

How we measure - currently available clinical methodologies Pro's and Con's

First, be aware that salivary or home salivary telomere testing is not yet available or FDA approved and is unlikely to become approved in the near future. There are essentially no scientifically validated studies around telomere length in saliva and the organism; all serious work to date has been performed in blood. Therefore, in my view, if your patient has done one of these tests, you must unfortunately disregard the results and use a more established proven method.
Q-PCR, uses a DNA amplification signal based on single copy genes. It is relatively inexpensive and likely to become cheaper and is fast

and well suited to studies with large populations of participants. It is not labor intensive and not particularly dependent on expertise and is logistically simple and scalable. It has been the standard used for large data base for comparison values and statistical machination and does not require living cells.

However, there are some very significant drawbacks. The degree of accuracy and reproducibility is listed as "undetermined" compared to other modalities. Values are extrapolated from signal intensity and not directly quantitative and it provides only average (mean) telomere length of the sample, is unable to quantify telomeres individually and therefore biologic age cannot be measured or inferred from this method.

FLO-FISH uses in situ hybridization technology. It is amenable to immunologic determination of lymphocyte profiles (example CD 4 and CD 8). It is more accurate, quantitative and reproducible than **Q-PCR**. However, it is more labor and time intensive and more costly. It is considered a less prominent but not insignificant research tool with good data base size. It does require live cells. But again, a major drawback is that it gives only average telomere length and not short telomere quantification or biologic age.

HT Q-FISH: Uses digital confocal microscopy to access large populations of individual telomeres. There is one lab which has been able to automate for high scalability while maintaining a high level of accuracy and reproducibility. This is becoming the new scientific standard as it is the only technology that can measure telomeres chromosome by chromosome and hence short telomeres which correlates to biologic age. In addition, due to greater sensitivity and accuracy, less time passage between testing is required so may be more useful for clinical interventions. Because it quantifies telomeres individually, it provides the much more useful median telomere length (although average telomere length can also be generated). It is, however, the most expensive assay currently should become more affordable over time. It does require live cells which make logistics more important and it is more labor and time intensive and requires a high level of expertise in the lab.

In conclusion, **HT Q-FISH** is the only technology that currently measures the presence of short telomeres which appears to be the most important consideration for "biological age."

When you measure, now what?

In order to address this, you need to understand the following:

1) A single isolated test of ANY KIND is of little value. Trends are established with 2 or more data points over time.

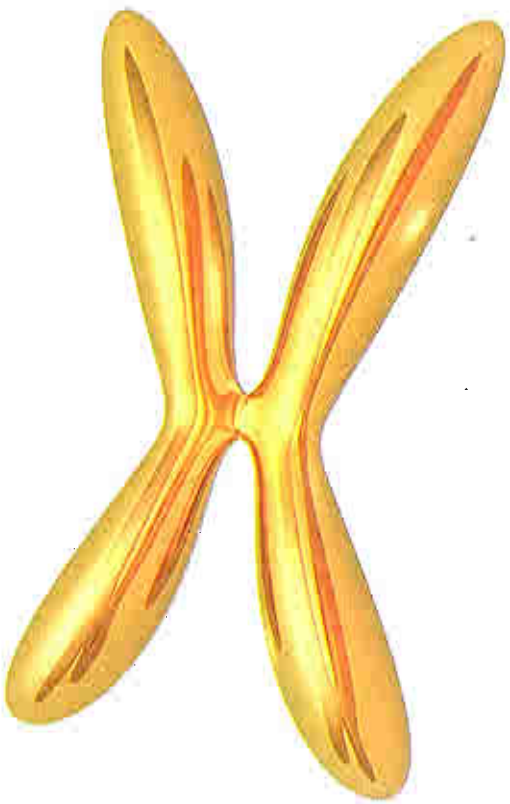
2) No one born before 2010 is likely to have any record of their telomere length at birth or any other time in life until you and they decide to measure it. Therefore you cannot ferret out who was born with long or short telomeres. The length of telomeres at birth is known to vary in healthy individuals based on many factors including parental age and ethnicity as well as transient and chronic physical and mental factors. This again stresses the importance of #1 directly above!

Be prepared for some surprises in your patient population. No matter what technology you decide on you will see "outliers" and some things that do not make sense especially with the initia measurement. More specifically you will find people who live we outside your recommendations who seem to have "good " telomer measurements and people who are highly compliant that hav "bad measurements." Address this the way you would any othe biomarker in the sense of getting your patient healthier. Use th information to make your patient an ally of yours (and theirs!) an get them to take even better care of themselves. Remind them an yourself that a single isolated telomere measurement is of little valu without doing another one down the road to establish a "trend" The more data points you have the better you will be able to assig prognostic value. Remind your patient and yourself.

If a patient was gifted with long telomeres and a great anti-oxidan system they may statically do better - but why not get them t take advantage of what they were given? Similarly if a patien was born with shorter telomeres and poor defenses, it would be prudent to get them to address any factors they can to mitigate the cellular damage that is manifesting as shorter than average (o shorter median) telomeres and an increased or increasing percentage of short telomeres correlating with an older biological age thar chronological age.

Analyze your patients' telomere results reports in the context of their family history and physical. The age of their grandparents and parents on both sides at the time of death is often a telling factor. The company that uses the HT Q-FISH technology has a detailed questionnaire to help you look at known and suspected "Telomere Risk Factors".

Above all else, follow this field and stay educated because all the signs are pointing to increased ability to intervene in a positive fashion for the overall health and wellness of your patient, and the concomitant need and importance of accurate testing!



Summary

The recent explosion of telomere based research has led to a much deeper understanding of the pivotal role telomere attrition plays in the aging process human and otherwise. The importance of longer healthier telomeres with the concomitant reduction in critically short telomeres that activate the DNA damage response and may prematurely age cells is no longer controversial.

Many of the diseases we associate with aging can be followed and prognosticated with telomere length in addition to more commonly accepted variables.

There are typical and atypical interventions that can mitigate telomere damage and in some cases actually extend telomere length (beyond the scope of this paper), that make telomere testing more than just an interesting number. We can anticipate as the data grows and the robustness of telomere length to disease and aging becomes more widely accepted, so too will telomere testing become far more common and eventually become a standard of care.

There are several different clinical tests available each differing in many critical aspects. When you chose understand that you will want a follow up value at some defined interval to establish a trend for you patient. Similarly if you initiate any interventions in lifestyle or long term pharmaceutical management it would be wise to consider a repeat telomere test at some interval from that intervention.

Finally remember you are at the vanguard of the "Anti-Aging Revolution." Take care of and measure your own telomeres!

About the Author

Dave Woynarowski MD is a Board certified Internist and Anti-Aging doctor who went into supplement and Nutraceutical design after 18 years of clinical medicine. He owns and operates his own website DrDave'sBest.com and has written frequent articles about Telomeres, Telomere Measurement Telomerase and TA-65. He is co-author of the book, "The Immortality Edge" with Dr Mike Fossel and Greta Blackburn and co-authored the TA-65 physician's manual with Dr. Fossel. A nationally known expert on telomeres he has lecutured at both AMMG and A4M on the topic as well as the Longevity Now conference in Costa Mesa, California. He is currently working on a second book on the topic of Telomeres, Telomerase activation and Longevity. Currently he serves as a consultant to Life Length Inc., RD Stem Cell Inc and is conducting parallel research with stem cells and telomerase activation.

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