Ursidae: The Undergraduate Research Journal at the University of Northern Colorado

Volume 5
Number 2 *McNair Special Issue*

Article 5

April 2019

Trichology: A Study of Hair and its Uses as Trace Evidence

Amanda Landron
University of Northern Colorado, land0197@bears.unco.edu

Trichology: A Study of Hair and its Uses as Trace Evidence

Amanda Landron
Mentor: Alan Price, MA., Criminal Justice

Abstract: The purpose of this research was to study the characteristics of hair for the use as trace evidence. These characteristics are being used to add validity and reliability from the use of hair to confirm the identity of a person. Eight different humans and one dog hair samples were collected and compared to identify a fictional suspect. All of the hairs, including the primary suspect's, were collected from a fictional crime scene. The characteristics were analyzed using a polarized microscope to microscopically compare the hairs and a ForensicGEM® DNA Extraction Kit, ZyGEM was used to extract DNA from the samples. The extracted DNA was then forwarded to the Biology Department at Colorado State University for sequencing. The hypothesis was that a positive identification would be made using this combination of microscopy and mitochondrial DNA extraction.

Keywords: *microscopy, hair analysis, DNA extraction, mitochondrial DNA*

Fictional Crime Scene

A female victim was found deceased in the trunk of an abandoned vehicle just outside the city limits. The body was partially wrapped in a painter's plastic drop sheet while her hands and feet were zip tied. The body was heavily bruised but the cause of death was anoxia resulting from a plastic bag zip tied around the victim's neck. No blood appeared to be present. Among the evidence found in the trunk and on the victim's body were nine different sets of hair, one belonging to a Saint Bernard. In this investigation, one of the samples was identified as the primary suspect's hair, so it was compared to the seven human hairs found within the crime scene. This associative evidence, or trace evidence, was used to place the suspect at the crime scene. It was also determined that the suspect owned a Saint Bernard.

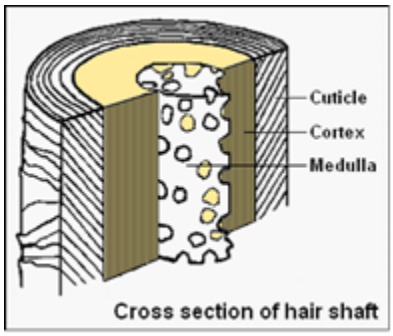
INTRODUCTION

When solving cases, crime scene investigators collect trace evidence. Trace evidence are materials that can be used to connect a victim, perpetrator, witness, and wildlife, to a crime scene. This association is called Locard's Exchange Principle. The concept is "every time you make contact with another person, place, or thing, it results in an exchange of physical material," (Locard's Exchange Principle, 2005, 428). To name a few types of physical material, there are DNA, fingerprints, footprints, skin cells, blood, bodily fluids, pieces of clothing fibers, and hair. I am focusing on how hair can be used as trace evidence. Trichology is the scientific study of hair. This is a study that demonstrates the use of trichology within criminal justice.

Thus far, there are three major types of hair analysis: macroscopic, microscopic, and chemical. Macroscopic analysis is looking at the hair with the naked eye. Microscopic analysis is looking at the different parts of the hair, and comparing them with a specialized microscope. The different parts of hair are the root, and shaft. The root is attached directly to the scalp, and although it contains the most amount of DNA, it is rarely found at a crime scene (Hampson, Louhelainen, & McColl, 2011). The shaft is composed of three layers: medulla, cortex, and cuticle (refer to Figure 1). The medulla contains mitochondrial DNA which can be extracted from the hair, analyzed, and may be used to identify an individual. The cortex surrounds the medulla and holds the pigment of the hair. The cuticle is the layer that coats the hair. These layers can be used to compare hairs to each other because they vary among all individuals and animal species. In chemical analysis, the hair is used to identify extraneous chemicals to which the individual was exposed to. Thus narrowing the list of possible locations the person has been. It is also used to extract DNA from the hair. This method,

although it reveals vital information that would not have been revealed otherwise is damaging to the hair. Therefore, this latter method is only used when large quantities of the hair are collected from a crime scene.

Figure 1. Cross section of hair shaft (Taylor, 2015)



Literature Review

Up until the last couple of decades, the validity of the use of hair as trace evidence in our judicial system has been the most challengeable evidence in court. With the recent discovery of new methods of analyzing trace evidence, however, the validity of trace evidence is improving. Trace evidence is used in the court room to connect a victim, suspect, or sometimes a witness to a crime scene and is referred as "associative evidence". Linking these connections allows crime scene investigators to successfully solve crimes. Houck (2004) discusses real life cases and how the use of trace evidence has successfully resolved cases.

Typically, hair at a crime scene may be one type of evidence. It has been found that the use of hair as trace evidence is very important when solving cases. The use of analyzing hair as trace evidence may answer the question of who committed the crime. Robertson and Roux (2010), state that focusing on the question of who did it will help with discovering how they did it, which in turn solves more cases. Furthermore, hair as trace evidence is easy to locate and is easy to analyze. Due to electrostatic forces, hairs attach themselves to surfaces very easily allowing the discovery of what the surroundings were of the person had been (Wiltshire, 2006).

Another significant importance hair has is that it can be found in many different forms. There is tonsorial hair (head hair), axillary hair (body hair), and pubic hair. Researchers have found that these different types of the hair may be used to identify or eliminate a suspect (Taupin, 1996; Burley, 2004; Lerner & Lerner, 2005). Some techniques that are used to analyze hair as trace evidence are with an x-ray microscope, which allows the observer to identify the spacing of the medulla from the cortex (Youn & Jung, 2005). The electron microscope can be used to obtain a 3-D image of the hair (Wagner, 2007). Along with the use of these microscopes, a computerized hair mapping system is used to compare hair samples side by side with pristine imaging (Verma & et al., 2002). Although these methods have been practiced repeatedly, the validity of the results of these methods are scrutinized. As past incidences have suggested, the results of these methods depends mostly on the examiner conducting the examination. In the past, suspects have been wrongly accused based on the justification of a forensic examiner (Neufeld, 2005). The goal of this research is to begin the process of improving the validity of the use of hair as trace evidence by becoming familiar with the process to accurately analyze hair.

Significance

Although hair is used as trace evidence, there is still controversy as to whether the use of hair is valid for obtaining conviction or achieving a pardoning. Due to research, however, it was found that mistakes such as these are primarily due to the examiner who did the analysis. They claimed to be positive, and used manipulated wording to erroneously lead juries astray (Giannelli, 2001). In a recent article, it was revealed that over 200 cases were falsely testified by the forensic examiners (Hsu, 2015). Some of the cases resulted in the death penalty. The death penalty is a severe sentence, and to falsely convict a perpetrator to their death using hair as trace evidence is not a scientific issue, but human error. It is possible that in the future, this research may be extended and focus on the person conducting the analytical methods and how to avoid misleading the jury.

Current Research

This research is a study on the characteristics of hair. Human and dog hair samples were collected and compared to a singular hair sample that was randomly selected as a fictional suspect. My hypothesis is that a positive identification may be made using a combination of microscopy and mitochondrial DNA extraction. The purpose of my research is to increase the validity and reliability by using hair to establish an individual's identity and strengthen the identity by moving from class characteristics to being individualistic. Also, I am hoping that this research will help the forensic science program at UNC to grow.

METHODS

Participants

I had eight participants volunteer to remove at least ten of their own hairs for me to analyze. I also obtained a hair from a Saint Bernard which was used to compare the class characteristics between human and dog hair. The volunteers came from an upper level classes, who were anonymous to everyone except my mentor, and the dog hair was obtained from a Saint Bernard dog by Professor Price. The volunteers were female, and age did not matter, as long as they were 18 years or older for legal purposes. The volunteers were all brunettes, with a variation of shades. IRB approval was obtained and a voluntary participation form was signed by each participant who provided a hair sample.

Hair collection and storage

The hairs were collected using gloves, a sanitized comb, and sterile envelopes. The envelopes were labeled as 3-C, 4-D, 5-E, 6-F, 7-G, 8-H, and 9-I, to keep identities anonymous. Once the hairs were collected, they were held in a chain of evidence custody drawer.

Procedures

Microscopy

To ensure the hairs remained separated and decontaminated, I mounted each hair one at a time. Paramount was used to permanently stick the hairs to the slides. The cases in which little hair is collected, xylene is the preferred mounting substance to use. Xylene has the same effect on increasing the ability to analyze hair under the microscope, without damaging the hair (Verma, 2015). Once the hairs were mounted and dried, each hair was analyzed and photographed. The photographs were then compared side by side, until the samples were reduced to four possible matches. I then repeated the mounting process with these four hairs to analyze a different section of the hairs. After comparing these photographs, I narrowed it down further to two possible hairs that could match the suspect, 3-C. I then remounted all three hairs to analyze yet another section of the hair, which resulted in feeling comfortable in moving on to the next step.

DNA Extraction

Director Keenan supervised me throughout the process and graciously allowed me to use her lab and materials that were needed. For the extraction a ZyGEM tissue kit was used. The kit had a 10x buffer, the extraction chemical, forensicGEM, and DNA-nase free water. DNA-nase water does not have DNA protein so as to not interfere with the DNA that was being extracted from the hair.

To prepare the hairs for the extraction, a two inch piece of hair was cut into tiny piece and placed in thin walled PCR tubes. Then, in this order, 89 µl of DNA-nase free water, 10 µl of 10x buffer, and 1 µl of forensicGeEM were also inserted into the tubes. With a total of three tubes, for the hairs 3-C, 7-G, and 9-I, they were placed in an incubator. For 15 min the temperature was 75°C, and then was maintained for five min at 95°C. During the first 15 min when the proteinase is activated, the cells were lysed, the nucleases destroyed, and the nucleoprotein is removed. The last 5 min is to inactivate the proteinase and the DNA is extracted and floating in the solution. To ensure that there was really DNA present, I tested the concentration of DNA in the hair on a NanoDrop 2000. I repeated the extraction to determine if a higher concentration can be obtained. The results were the same.

Polymerase Chain Reaction

Polymerase chain reaction is an instrument that is used to amplify and repeat a short sequence found on DNA. To amplify the sequence, primers are used to target the desired sequence. For the purpose of this research, the primers used were hypervariable region I F15975, R16418, and hypervariable region II, F15, R429. The letters F and R mean forward and reverse. Thus applies to the numbers and the location of the sequence on the DNA that is being amplified. To prepare these primers, which are dried, they were liquefied and made stronger by adding DEPC water.

Then, using a LifeTechnologies Kit, I prepared six different tubes of water, buffer, magnesium chloride, the template DNA, primers dNTPS, and taq polymerase, refer to Table 1. When determining how much of a concentration of the template DNA to use, it was difficult due to existing papers excluding this information. For this experiment, the director of the Biology department used her best judgment and we decided to use a 200nm concentration of DNA from the hair extraction (boxed in red on Table 1).

Table 1. PRC routine

	HV I			HV II		
	3-C	7-G	9-I	3-C	7-G	9-I
1. H_2O (µl)	30.6	31.9	30.9	30.6	31.9	30.9
2. 10x Buffer (µl)	5	5	5	5	5	5
3. 50 mM $MgCl_2$ (1.5 mM) (µl)	1.5	1.5	1.5	1.5	1.5	1.5
4. Template DNA (200 nm) (µl)	6.7	5.4	6.8	6.7	5.4	6.8
5. 10x Primers (µl)	5	5	5	5	5	5
6. dNTPs (.2mM) (µl)	1	1	1	1	1	1
7. Taq P (µl)	.2	.2	.2	.2	.2	.2
Total (µl)	50	50	50	50	50	50

Once the solutions were prepared, there were incubated in a Biorad T100 Thermocycler, using the following procedure: 94°C for 2minutes, then 38 cycles of 94°C for 30 seconds; 60°C for 30 seconds; 72°C for seconds; 72°C for 10 minutes. Then at 12°C for a holding phase. The total time of incubation was an hour and 42 minutes.

To ensure that the regions were successfully sequenced, I ran an electrophoresis gel of the samples. To prepare the gel, 1 gram of agarose powder was mixed and heated with 50mL of liquid TAE buffer. Once the gel solution was cooled, the first well was then loaded with a ladder, to measure the distance each sequence travels through the gel, and the other six solutions. The gel was then run for nearly 2 hours at 60 volts.

Sequencing

Since hypervariable region one did not show complete results, only samples for hypervariable region two were sent. These samples were prepared as per the parameters that the CSU biology department required. The process involved diluting the PCR prepared samples and the primers to a 1:10 ration using autoclaved water.

RESULTS

Microscopy

After repeating the process three times, the probable matches to the suspect seemed to be 7-G or 9-I, based on their physical characteristics. Figures 2, 3, and 4 indicate a strong similarity of either the hair color or pattern of the medulla. The other samples did not share significantly strong similarities and were therefore ruled out as possible matches to the suspect.

Figure 2. Microscopic view of hair sample 7-G

Figure 3. Microscopic view of hair sample 3-C

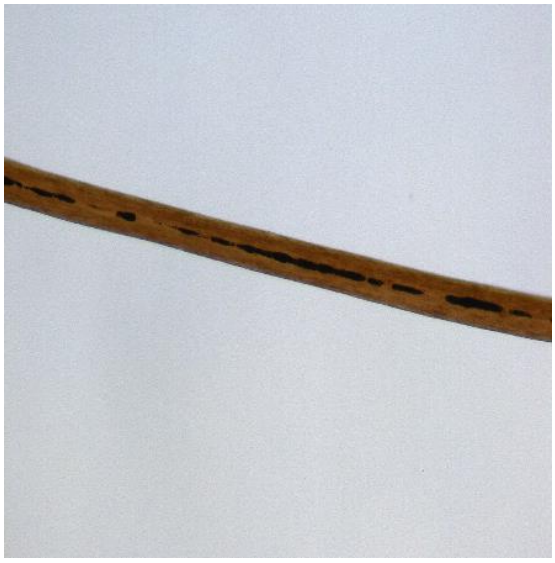


Figure 4. Microscopic view of hair sample 9-I

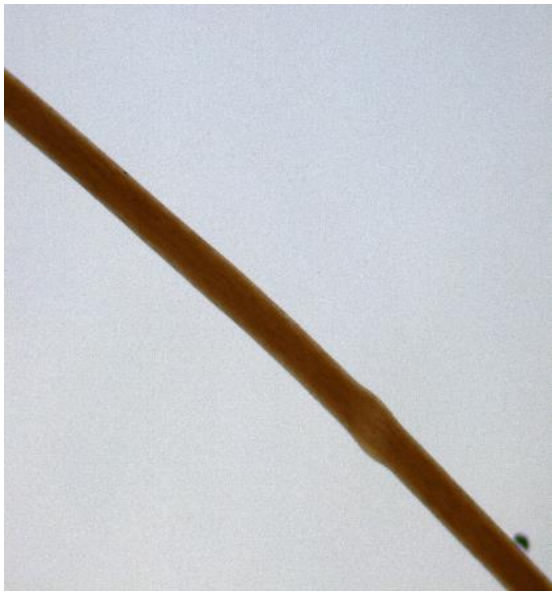
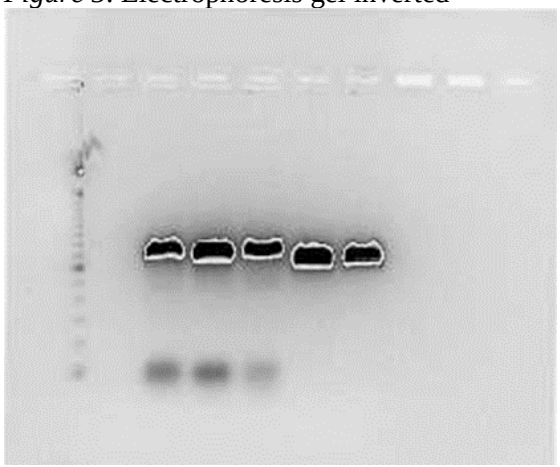


Figure 5. Electrophoresis gel inverted



DNA Extraction

The follicle, or root, was not used, since not all samples had the root attached. The amount of DNA extracted was calculated by determining concentration (Table 1). The DNA was extracted twice, with two different pieces of hair for each, to determine whether a high concentration can be obtained. After considering the amount of hair being used and only using mitochondrial DNA, it was decided that the concentration was exceptionally high.

Polymerase Chain Reaction

The gel showed a couple of discrepancies (Figure 5). Not only was one of the bands missing from the six that were hoped for, but the dimers were missing for three of the samples as well. The bands indicated that there was DNA present in the sample, and the dimers were the remnants of the primers that were used. The results indicated that 3-C did not have a trace of HVII and it is unclear as to why the dimer regions did not appear across the gel. There was also an issue with the dyeing of the ladder because it did not appear as clear as it should have. The positive result of the PCR, however, was that the extraction was successful with at least one primer. Considering, only HVII was transferred to CSU for sequencing.

Sequencing

The prepared samples were sent to the Biology Department at Colorado State University. The results displayed in Table 2 were unsatisfying. For the samples that did have a result, the shortened sequence could not be established due to the sequences being incomplete. Also, no results were returned from the suspect samples. Furthermore, preventing even partial matches to be made.

Table 2. HVII sequence

Sample	HVII
Primer	F15
3-C	NA
7-G	CAAACCGAAGGGGGCCATCTCTGTGGTGTGAGGGGGGGGTCAGGCGGTAACCAAAGGGGAAGGACCCTCAATTTTACAGGCGAACATCC TTACTAAAGTGTGTTAATTAATTAATGTCTTGTTGGACTTCCCTAATAAACAATTGAATGTCTGCACAGACGCTTTCCACACAGACAGTCGAAA CAAAAAATTTCCACCATACCCCCCCCCTCCTCCCGGTTCTGGGCACGAATTTAACCAAAGTACTGCCGAACCCTAGGGGAAAAAGAACGGTA CCCCGGCCTAATTTGAATTCCAATATATAAA
9-I	CAGGGAAGGGGGGGCCTAGTTGTGGTTTAGGGGGGGTTGTGGATTCCAAGGGAAGGGGTGTGTGCAAGGTAGGGCGAACATACTTATT GAAGGGTGTTATTTAGTAAAGTTGTGTTACAAATACAATTGATTCGTCGAGTTATGAAAGGGAAAAATTCACAAGCCATTCACGTTAGGAAAAAG TACAGCTCGGAACCAAGAAAGTCGTAACGGGT

DISCUSSION

Based on current findings, to successfully identify a suspect via extracting DNA from hair samples is precise and difficult, but possible. DNA has been successfully extracted and analyzed with only the use of approximately 2 inches of hair. In future experiments, these techniques should be practiced prior to starting. By further extension, future research should include, but need not be limited to, identifying what chemicals will cause the presence of DNA within the hair follicle to disappear. This type of experiment can be used to determine whether or not DNA can still be recovered from hair collected at a crime scene. Therefore, the significance of this research is to improve the collection and analyses of hair as trace evidence.

Case Solved?

Unfortunately, this case is still open. Due to impartial and insufficient evidence, we were unable to place the suspect at the scene of the crime. This is evident, however, that methods and processes of analyzing trace evidence still has room to grow. Hopefully in future cases, success will be evident all across the board.

REFERENCES

Acevedo, S.F. (2012). Why, after chemotherapy, is it necessary to assess memory using translational testing? *Breast Cancer: Basic and Clinical Research, 6*, 181-190.

Ahles, T.A. (2012). Brain vulnerability to chemotherapy toxicities. *Pshyco-Oncology, 21*, 1141-1148. doi: 10.1002/pon.3196

Ahles, T.A., & Saykin, A.J. (2007). Candidate mechanisms for chemotherapy-induced cognitive changes. *Nature Reviews Cancer, 7*, 192-201. doi: 10.1038/nrc2073

American Cancer Society. (2014). Cancer facts and figures 2014. Retrieved from http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2014

Arnold, R., Rinke, A., Schmidt, Ch., & Hofbauer, L. (2005). Chemotherapy. *Best Practice & Research Clinical Gastroenterology, 19*, 649-656.

Baker, F., Denniston, M., Smith, T., & West, M.M. (2005). Adult cancer survivors: how are they faring? *Cancer, 104*, 2565-2576.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. *Archives of General Psychiatry, 4*, 561-571.

Belvoir Media Group, LLC, (2010). Chemotherapy. *Harvard Health Publication, Health Topics A-Z.*

Conroy, S.K., McDonald, B.C., Smith, D.J., Moser, L.R., West, J.D., Kamendulis, L.M.,… & Saykin, A.J. (2013). Alterations in brain structure and function in breast cancer survivors: effect of post-chemotherapy interval and relation to oxidative DNA damage. *Breast Cancer Research and Treatment, 137,* 493-502.

Courneya, K.S., & Karvinen, K.H. (2007). Exercise, aging, and cancer. *Applied Physiology, Nutrition, and Metabolism, 32*, 1001-1007.

Ferrans, C. E., & Powers, M. J. (1985). Quality of life index: development and psychometric properties. *Advances in nursing science, 8*(1), 15-24.

Grady, C.L. (2008). Cognitive neuroscience of aging. *Annals of the New York Academy of Sciences, 1124*, 127-144.

Hsieh, C.C., Sprod, L.K, Hydock, D.S., Carter, S.D., Hayward, R., & Schneider, C.M. (2008). Effects of a supervised exercise intervention on recovery from treatment regimens in breast cancer survivors. *Oncology Nursing Forum, 35*, 909-915.

Jackson, A. S., & Pollock, M. L. (1978). Generalized equations for predicting body density of men. *British Journal of Nutrition, 40*, 497-504.

Jackson, A. S., & Pollock, M. L. (1980). Generalized equations for predicting body density of women. *Medicine and Science in Sports and Exercise, 12,* 175-181.

Jemal, A., Center, M. M., DeSantis, C., & Ward, E. M. (2010). Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiology Biomarkers & Prevention, 19,* 1893-1907.

Koppelmans, V., de Ruiter, M.B., van der Lijn, F., Boogerd, W., Seynaeve, C., van der Lugt, A., & Schagen, S. B. (2011). Global and focal brain volume in long-term breast cancer survivors exposed to adjuvant chemotherapy. *Breast Cancer Research and Treatment, 132,* 1099-1106.

Mackenzie, L.J., Carey, M.L., Sanson-Fisher, R.W., & D'Este, C.A. (2013). Psychological distress in cancer patients undergoing radiation therapy treatment. *Support Cancer Care, 21,* 1043-1051.

Maggio, M., Dall'aglio, E., Lauretani, F., Cattabiani, C., Ceresini, G., Caffarra, P., & Ceda, G.P. (2012). Hormonal dysregulation and cognition in the elderly: The hormonal pathway to cognitive impairment in older men. *The Journal of Nutrition, Health & Aging, 16*(1), 40-54.

Piper, B.J., Acevedo, S.F., Craytor, M.J., Murray, P.W., & Raber, J. (2010). The use and validation of the spatial navigation memory island test in primary school children. *Behavioral Brain Research,* 210, 252-262.

Piper, B. F., Dibble, S. L., Dodd, M. J., Weiss, M. C., Slaughter, R. E., & Paul, S. M. (1998, May). The revised Piper Fatigue Scale: psychometric evaluation in women with breast cancer. *Oncology Nursing Forum*, 25, 677-684.

Pinto, B. M., Dunsiger, S., & Waldemore, M. (2013). Physical activity and psychosocial benefits among breast cancer patients. *Psycho-Oncology,* 22, 2193–2199. doi:10.1002/pon.3272

Proctor, R.N., (2003). Cancer. *Dictionary of American History*. 2 (3rd ed.), 34-35.

Quist, M., Rorth, M., Zacho, M., Andersen, C., Moeller, T., Midtgaard, J., and Adamsen, L., (2006). High-intensity resistance and cardiovascular training improve physical capacity in cancer patients undergoing chemotherapy. *Scandinavian Journal of Medicine and Science in Sports*. 16, 349-357.

Salkind, M. R. (1969). Beck depression inventory in general practice. *The Journal of the Royal College of General Practitioners, 18*, 267.

Schneider, C.M., & Hayward R. (2013). Cancer rehabilitation and cancer-related fatigue.

Journal of Clinical Exercise Physiology, 2(1), 1-7.

Schneider, C.M., Hsieh, C.C., Sprod, L.K., Carter, S.D., & Hayward, R. (2007). Cancer treatments-induced alterations in muscular fitness and quality of life: the role of exercise training. *Annals of Oncology, 18,* 1957-1962.

Schmitz, K.H., & Speck, R.M. (2010). Risks and benefits of physical activity among breast cancer survivors who have completed treatment. *Future Science Group, 6,* 221-238.

Schuurs, A., & Green, H.J., (2013), A feasibility study of group cognitive rehabilitation for cancer survivors: enhancing cognitive function and quality of life. *Psycho-Oncology*, 22, 1043-1049.

Siegel, R., DeSantis, C., Virgo, K., Stein, K., Mariotto, A., Smith, T.,… & Ward, E. (2012). Cancer treatment and survivorship statistics, 2012. *CA: A Cancer Journal for Clinicians, 62*, 220-241.

Summers, L. E. Effects of chemotherapy on patients' cognitive function. *Cancer Nursing Practice, 11*, 27-31.

Ward, E., DeSantis, C., Robbins, A., Kohler, B., & Jemal, A. (2014). Childhood and adolescent cancer statistics, 2014. *CA: A Cancer Journal for Clinicians, 64*, 83-103.

Wechsler, D. (2009). *Wechsler Memory Scale—Fourth Edition (WMS-IV) technical and interpretive manual*. San Antonio, TX: Pearson.

Wechsler, D. (2008). *Wechsler Adult Intelligence—Fourth Edition (WAIS-IV).* San Antonio, TX: Pearson.

Wefel, J.S., & Schagen, S.B. (2012). Chemotherapy-related cognitive dysfunction. *The Canadian Veterinary Journal, 50,* 665-668.

Weis, J., Poppelreuter, M., & Bartsch, H. H. (2011). Rehabilitation of therapy-related cognitive deficits in patients with breast cancer. *Psicooncología, 8,* 371-384.

Wolinsky, F.D., Unverzagt, F.W., Smith, D.M., Jones, R., Stoddard, A., & Tennstedt, S.L. (2006). The active cognitive training trial and health-related quality of life: protection that lasts for 5 years. *Journal of Gerontology: Medical Science, 61A* (12), 1324-1329.

Wolman, R.L., Cornall, C., Fulcher, K, & Greenwood, R. (1994). Aerobic training in brain-injured patients. *Clinical Rehabilitation, 8,* 253-257.