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14. ABSTRACT

Purpose: The purpose of this pilot study was to determine the sensitivity and specificity of small animal Positron Emission Tomography-Computed Tomography (PET-CT) in identifying metabolic changes in muscle tissue surrounding simulated shrapnel injuries and compare this imaging to traditional x-ray images. **Design**: Experimental design with repeated measures **Methods**: Fischer 344 male rats randomly assigned to three groups, were implanted with weapons grade heavy metal tungsten alloy (HMTA) pellets, tantalum (Ta) pellets as the control metal or Sham control without pellet implantation. Rats from each metal category received a series of x-rays and ^{18}F fluoro-2-deoxy-D-glucose (FDG) PET-CT scans over 16 weeks. Sacrificed animals at each of five time points over a 16 week period had tissue excised for histopathological examination. **Sample**: 32 Fischer 344 male rats (2 Sham , 15 Ta, 15 HMTA) **Analysis**: .Standardized uptake value (SUV) tracer uptake was quantified using the. Image data comparisons were accomplished using Kolmogorov-Smirnov Z, Friedman's ANOVA and Wilcoxon signed-rank tests. Sensitivity and specificity were determined. Receiver Operating Characteristic (ROC) curve and the area under the curve (AUC) were calculated. Significance level was set at $p < .05$. Histopathology was assessed by a pathologist, blinded to treatment groups. **Findings**: Increased FDG uptake was associated with an aggressive malignancy in the HMTA implanted rats. There was a significant difference in tracer uptake between the Ta and HMTA animals and also in tracer uptake over the sixteen weeks for the HMTA animals. PET-CT imaging had a sensitivity of 86%, specificity of 100% and AUC .938. **Implications for Military Nursing**: Military nurses have a unique opportunity to educate patients and providers about the possibility of early tissue changes around embedded fragments and the use of PET-CT imaging as a possible surveillance tool. When retained shrapnel is located, monitor patients for fragment migration and tissue changes.

15. SUBJECT TERMS

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Abstract

Purpose: The purpose of this pilot study was to determine the sensitivity and specificity of small animal Positron Emission Tomography-Computed Tomography (PET-CT) in identifying metabolic changes in muscle tissue surrounding simulated shrapnel injuries and compare this imaging to traditional x-ray images.

Design: Experimental design with repeated measures

Methods: Fischer 344 male rats randomly assigned to three groups, were implanted with weapons grade heavy metal tungsten alloy (HMTA) pellets, tantalum (Ta) pellets as the control metal or Sham control without pellet implantation. Rats from each metal category received a series of x-rays and ^{18}F fluoro-2-deoxy-D-glucose (FDG) PET-CT scans over 16 weeks. Sacrificed animals at each of five time points over a 16 week period had tissue excised for histopathological examination.

Sample: 32 Fischer 344 male rats (2 Sham , 15 Ta, 15 HMTA)

Analysis: Standardized uptake value (SUV) tracer uptake was quantified using the. Image data comparisons were accomplished using Kolmogorov-Smirnov Z, Friedman's ANOVA and Wilcoxon signed-rank tests. Sensitivity and specificity were determined. Receiver Operating Characteristic (ROC) curve and the area under the curve (AUC) were calculated. Significance level was set at $p < .05$. Histopathology was assessed by a pathologist, blinded to treatment groups.

Findings: Increased FDG uptake was associated with an aggressive malignancy in the HMTA implanted rats. There was a significant difference in tracer uptake between the Ta and HMTA animals and also in tracer uptake over the sixteen weeks for the HMTA animals. PET-CT imaging had a sensitivity of 86%, specificity of 100% and AUC .938.

Implications for Military Nursing: Military nurses have a unique opportunity to educate patients and providers about the possibility of early tissue changes around embedded fragments and the use of PET-CT imaging as a possible surveillance tool. When retained shrapnel is located, monitor patients for fragment migration and tissue changes.

TSNRP Research Priorities that Study or Project Addresses

Primary Priority

Force Health Protection:	☐ Fit and ready force ☐ Deploy with and care for the warrior ☒ Care for all entrusted to our care
Nursing Competencies and Practice:	☐ Patient outcomes ☐ Quality and safety ☐ Translate research into practice/evidence-based practice ☐ Clinical excellence ☐ Knowledge management ☐ Education and training
Leadership, Ethics, and Mentoring:	☐ Health policy ☐ Recruitment and retention ☐ Preparing tomorrow's leaders ☐ Care of the caregiver
Other:	☐

Progress Towards Achievement of Specific Aims of the Study or Project
Findings related to each specific aim, research or study questions, and/or hypothesis:

Findings related to each specific aim

1. To investigate changes in inflammation over time after heavy metal tungsten alloy (HMTA) pellets are embedded in the muscles of F344 rats using ^{18}F-FDG PET-CT imaging.

   The mean cross sectional area of the muscle fibers, number of muscle fibers and nuclei in the soleus, plantaris, medial and lateral gastrocnemius muscles of the euthanized rats were compared at each of the five time points (see Table 1).

Table 1: *Mean Cross Sectional Area of Muscle fibers, Muscle Fiber and Nuclei Counts*

Week 1						
	TA n = 2					
Muscle	Muscle wt(mg)	Muscle wt (mg)/bw (g)	Sample Fibers	Fiber CSA µm²	Sample Nuclei	Nuclei /Fiber
Soleus	85 ± 5	.35 ± .01	173 ± 3	951 ± 7.7	673 ± 9	4 ± .01
Plantaris	190 ±10	.82 ± .03	180 ± 13	953 ± 73.2	602 ± 42	3 ± .01
Gastrocnemius M			166 ± 10	1023 ± 270.9	508 ± 4	3 ± .02
Gastrocnemius L			168 ± 3	1186 ± 19.1	575 ± 6	3 ± .09
Gastrocnemius	1175 ± 35	4.83 ± .01				
	HMTA n = 2					
Muscle	Muscle wt(mg)	Muscle wt (mg)/bw(g)	Sample Fibers	Fiber CSA µm²	Sample Nuclei	Nuclei /Fiber
Soleus	85 ± 5	.37 ± .03	171 ± 7	977 ± 24.5	637 ± 29	4 ± .32
Plantaris	190 ± 10	.82 ± .05	181 ± 14	924 ± 64.8	598 ± 44	3 ± .02
Gastrocnemius M			163 ± 3	1258 ± 18.3	539 ± 75	3 ± .52
Gastrocnemius L			179 ± 23	1073 ± 19.2	473 ± 88	3 ± .15
Gastrocnemius	1075 ± 45	4.62 ± .14				

Note. wt- weight; mg – milligram; bw – body weight; g – gram; mean ± standard error of the mean were annotated for the CSA µm² and the number of nuclei per fiber; CSA – Cross sectional area; µm micrometer; (M) – medial head of the muscle; (L) – lateral head of the muscle; weight for the gastrocnemius muscle is for the entire muscle (medial and lateral heads)

Table 1: Continued: *Mean Cross Sectional Area of Muscle fibers, Muscle Fiber and Nuclei Counts*

Week 7						
	TA n = 2					
Muscle	Muscle wt(mg)	Muscle wt (mg)/bw (g)	Sample Fibers	Fiber CSA µm²	Sample Nuclei	Nuclei /Fiber
Soleus	125 ± 5	.39 ± .01	157 ± 5	1377 ± 10.7	621 ± 28	4 ± .06
Plantaris	270 ± 20	.84 ± .05	174 ±14	1141 ± 19.1	517 ± 78	3 ± .22
Gastrocnemius M			164 ± 11	1975 ± 520	599 ± 64	4 ± .64
Gastrocnemius L			168 ± 6	1630 ± 118	637 ± 92	4 ± .67
Gastrocnemius	1475 ± 5	4.56 ± .09				
	HMTA n = 2					
Muscle	Muscle wt(mg)	Muscle wt (mg)/bw (g)	Sample Fibers	Fiber CSA µm²	Sample Nuclei	Nuclei /Fiber
Soleus	115 ± 5	.34 ± .01	154 ± 3	1311 ± 148	633 ±14	4 ± .01
Plantaris	310 ± 20	.92 ± .03	169 ± 16	1165 ± 64.5	495 ± 52	3 ± .04
Gastrocnemius M			177 ± 1	1231 ± 23.4	537 ± 13	3 ± .09
Gastrocnemius L			156 ± 1	1642 ± 20.3	570 ± 15	4 ± .12
Gastrocnemius	1645 ± 15	4.89 ± .18				

Week 10						
	TA n = 2					
Muscle	Muscle wt(mg)	Muscle wt (mg)/bw (g)	Sample Fibers	Fiber CSA µm²	Sample Nuclei	Nuclei /Fiber
Soleus	145 ± 5	.39 ± .01	174 ± 3	1526 ± 47.6	767 ± 9	4 ± .11
Plantaris	370 ± 20	1.00 ± .01	176 ± 0	1381 ± 90.7	683 ± 10	4 ± .05
Gastrocnemius M			167 ±15	1562 ± 105	579 ± 21	4 ± .44
Gastrocnemius L			154 ± 3	1813 ± 163	582 ± 6	4 ± .02
Gastrocnemius	1810 ± 90	4.87 ± .04				
	HMTA n = 2					
Muscle	Muscle wt(mg)	Muscle wt (mg)/ bw (g)	Sample Fibers	Fiber CSA µm²	Sample Nuclei	Nuclei /Fiber
Soleus	125 ± 15	.33 ± .02	167 ± 9	1480 ± 68.7	749 ± 35	5 ± .04
Plantaris	345 ± 5	.92 ± .05	170 ± 1	1238 ± 151	558 ± 28	3 ± .17
Gastrocnemius M			160 ± 6	1388 ± 212	564 ± 67	4 ± .29
Gastrocnemius L			168 ± 5	1639 ± 378	587 ± 5	3 ± .14
Gastrocnemius	1920 ± 120	5.09 ± .09				

Week 13						
	TA n = 2					
Muscle	Muscle wt(mg)	Muscle wt (mg)/bw (g)	Sample Fibers	Fiber CSA µm²	Sample Nuclei	Nuclei /Fiber
Soleus	125 ± 25	.32 ± .05	167 ± 3	1702 ± 72.6	668 ± 15	4 ± .03
Plantaris	325 ± 25	.85 ± .01	162 ± 12	1484 ± 14.9	599 ± 61	4 ± .10
Gastrocnemius M			162 ± 12	1487 ± 114	572 ± 3	3 ± .02
Gastrocnemius L			160 ± 4	1395 ± 6.1	533 ± 18	3 ± .02
Gastrocnemius	1735 ± 85	4.55 ± .05				
	HMTA n = 2					
Muscle	Muscle wt(mg)	Muscle wt (mg)/bw (g)	Sample Fibers	Fiber CSA µm²	Sample Nuclei	Nuclei /Fiber
Soleus	110 ± 30	.30 ± .07	161 ± 6	1527 ± 88.6	683 ± 43	4 ± .12
Plantaris	330 ± 30	.90 ±.06	160 ± 8	1432 ± 72.2	594 ± 35	4 ± .40
Gastrocnemius M			171 ± 10	1333 ± 64.8	555 ± 6	3 ± .16
Gastrocnemius L			168 ± 2	1797 ± 174	641 ± 15	4 ± .04
Gastrocnemius	1650 ± 30	4.48 ± .04				

Note. wt- weight; mg – milligram; bw – body weight; g – gram; mean ± standard error of the mean were annotated for the CSA µm² and the number of nuclei per fiber; CSA – Cross sectional area; µm micrometer; (M) – medial head of the muscle; (L) – lateral head of the muscle; weight for the gastrocnemius muscle is for the entire muscle (medial and lateral heads)

Table 1 Continued: *Mean Cross Sectional Area of Muscle fibers, Muscle Fiber and Nuclei Counts*

Week 16 n = 4

	Sham Control n = 2					
Muscle	Muscle wt(mg)	Muscle wt (mg)/bw (g)	Sample Fibers	Fiber CSA µm²	Sample Nuclei	Nuclei /Fiber
Soleus	120 ± .0	.33 ± .01	166 ± 9	1402 ± 52.2	701 ± 65	4 ± .17
Plantaris	355 ± 5	.98 ± .02	171 ± 5	1460 ± 86.5	659 ± 76	4 ± .34
Gastrocnemius M			155 ± 4	1802 ± 199	578 ± 17	4 ± .02
Gastrocnemius L			158 ± 1	1482 ± 206	543 ± 38	3 ± .26
Gastrocnemius	1705 ± 15	4.71 ± .12				
	Ta n = 2					
Muscle	Muscle wt(mg)	Muscle wt (mg)/bw (g)	Sample Fibers	Fiber CSA µm²	Sample Nuclei	Nuclei /Fiber
Soleus	133 ± 4.5	.35 ± .01	166 ± 7	1737 ± 38.9	786 ± 62	5 ± .18
Plantaris	340 ± 7.6	.89 ± .02	162 ± 11	1675 ± 129	611 ± 5	4 ± .29
Gastrocnemius M			167 ± 10	1518 ± 202	590 ± 48	4 ± .09
Gastrocnemius L			162 ± 7	1821 ± 259	646 ± 61	4 ±.54
Gastrocnemius	1761 ± 25	4.59 ± .09				
	HMTA n = 2					
Muscle	Muscle wt(mg)	Muscle wt (mg)/bw (g)	Sample Fibers	Fiber CSA µm²	Sample Nuclei	Nuclei /Fiber
Soleus	140 ± 8.2	.36 ± .02	162 ± 4	1394 ± 10.9	680 ± 2	4 ± .10
Plantaris	357 ± 11	.92 ± .03	168 ± 2	1843 ± 53.4	671 ± 19	4 ± .16
Gastrocnemius M			165 ± 7	1807 ± 143	613 ± 86	4 ± .38
Gastrocnemius L			162 ± 7	2021 ± 204	646 ± 39	4 ± .07
Gastrocnemius	1754 ± 22	4.55 ± .06				

Note. wt- weight; mg – milligram; bw – body weight; g – gram; mean ± standard error of the mean were annotated for the CSA µm² and the number of nuclei per fiber; CSA – Cross sectional area; µm micrometer; (M) – medial head of the muscle; (L) – lateral head of the muscle; weight for the gastrocnemius muscle is for the entire muscle (medial and lateral heads)

Blood component cell numbers and shapes are indicators of health status. Increases in white blood cell counts can indicate inflammation. Complete blood counts were a comparative measure of inflammation. Only two viable blood specimen were obtained from week one (one Ta, one HMTA) and lab results were very similar for the two animals with the exception of the difference in the number of platelets (see Table 2). The HMTA animal had a high value of 624 for platelets. This was higher than compared to the 489 platelet count for the sham control but only slightly higher than the normal platelet value for rats.

Table 2:
Hematology Parameters for Euthanized Rats from Week One

	Week 1 Post Implantation	
	Ta	HMTA
White blood cells (10^3/mm^3)	3.4	4.0
Red blood cells (10^6/mm^3)	8.33	8.15
Hemoglobin (g/dl)	16.0	15.2
Hematocrit (%)	44.3	43.2
MCV (fl)	53.0	53.0
MCH (pg)	19.2	18.6
MCHC (g/dl)	36.2	35.1
RDW (%)	13.6	12.5
Platelets (10^3/mm^3)	489.0	624.0
MPV (fl)	5.9	5.9
Lymphocytes (10^3/mm^3)	1.4	1.8
Monocytes (10^3/mm^3)	0.4	0.5
Granulocytes (10^3/mm^3)	1.6	1.7

Note. Data from week 1 contained one Ta observations and one HMTA observations (one Ta and one HMTA specimen were discarded. MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC- mean corpuscular hemoglobin concentration; RDW – red blood cell distribution width; MPV – mean platelet volume; Ta- Tantalum implanted animals; HMTA – Heavy Metal Tungsten Alloy implanted animals.

Kolmogorov-Smirov Z tests determined no statistically significant differences in any of the hematological values measured between the Ta and HMTA groups for the first four time points (week 7, 10 and 13). Statistical significance was at $p < .05$. The Kruskal-Wallis Test was performed to compare the sham control (n = 2), Ta (n = 8) and HMTA (n = 9) groups for final time point (see Table 3).

Table 3:
Kruskal-Wallis Test (Sham, Ta, and HMTA) Hematological Comparisons From Week 16

	Sham	Ta	HMTA		
	X ± SEM	X ± SEM	X ± SEM	H(*df*)	*p*
WBCs ($10^3/mm^3$)	5.35 ± 0.50	3.79 ± 0.23	3.89 ± 0.27	5.31(2)	.070
RBCs ($10^6/mm^3$)	9.06 ± 0.24	8.47 ± 0.07	8.17 ± 0.09	10.15(2)	. 006*
Hemoglobin (g/dl)	15.70 ± 0.20	14.95 ± 0.11	14.94 ± 0.15	4.57(2)	.102
Hematocrit (%)	45.60 ± 1.20	43.85 ± 0.51	43.20 ± 0.48	3.73(2)	.155
MCV (fl)	50.00 ± 0.00	51.88 ± 0.35	52.89 ± 0.31	8.42(2)	.015*
MCH (pg)	17.35 ± 0.15	17.65 ± 0.12	18.29 ± 0.14	7.39(2)	.025*
MCHC (g/dl)	34.45 ± 0.45	34.14 ± 0.31	34.60 ± 0.30	1.55(2)	.462
RDW (%)	14.20 ± 0.40	14.26 ± 0.24	13.64 ± 0.13	6.06(2)	.048*
Platelets ($10^3/mm^3$)	572.50 ± 52.50	503.13 ± 31.75	523.44 ± 25.27	1.26(2)	.532
MPV (fl)	6.60 ± 0.10	6.30 ± 0.06	7.01 ± 0.11	12.49(2)	.002*
Lymphocytes ($10^3/mm^3$)	2.40 ± 0.30	1.61 ± 0.15	1.63 ± 0.15	4.06(2)	.132
Monocytes ($10^3/mm^3$)	0.60 ± 0.00	0.38 ± 0.04	0.41 ± 0.04	4.67(2)	.097
Granulocyte ($10^3/mm^3$)	2.35 ± 0.25	1.80 ± 0.09	1.84 ± 0.11	4.23(2)	.121

Note. Data represents the mean (X) ± standard error of the mean (SEM).Data from week 16 contained eight Ta, nine HMTA and two sham control observations (N = 19 Sham control n = 2, Ta n = 8, HMTA n = 9); WBCs – white blood cells; RBCs; red blood cells MCV– mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC- mean corpuscular hemoglobin concentration; RDW – red blood cell distribution width; MPV– mean platelet volume; Ta- Tantalum implanted animals; HMTA– Heavy Metal Tungsten Alloy implanted animals; H – test statistic for the Kruskal-Wallis test; *df* – degrees of freedom; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC- mean corpuscular hemoglobin concentration; RDW – red blood cell distribution width; MPV – mean platelet volume; Ta- Tantalum implanted animals; HMTA – Heavy Metal Tungsten Alloy implanted animals; Sham Control – animals had surgery without any metal pellets implanted; *p* – significance; * - significance is $p < .05$

There was a statistically significant difference in RBC (H(2) = 10.15, p = .006), MCV (H(2) = 8.42, p = .015), MCH (H(2) = 7.39, p = .025), RDW (H(2) = 6.06, p = .048) and MPV (H(2) = 12.49, p = .002) of the hematological values between the sham control, Ta and HMTA animals for week 16. Mann –Whitney tests were used as post hoc tests with a Bonferroni correction and a critical value for significance at p <.017. There were no significant differences between the sham control and the Ta animals or the sham and HMTA animals for any of the hematological parameters measured (p > .017). All animal hematological values were within normal limits with the exception of the RDW which was low for all of the animals.

C reactive protein offers a measure of inflammation in for cardiovascular disease. The rat serum was tested for C reactive protein differences between the Sham, TA and HMTA groups using an Enzyme-Linked Immunosorbent Assay (ELISA) (ABCAM, Cambridge, MA). The results were inconclusive due to the lack of a standard curve to measure assay results from.

2. To determine the sensitivity and specificity of using 18F-FDG PET-CT imaging as a marker of chronic inflammation in tissue surrounding an embedded metal fragment (HMTA) known to cause aggressive rhabdomyosarcomas in rats.

3. To determine the sensitivity and specificity of using 18F-FDG PET-CT imaging as a biomarker of tumor formation in tissue surrounding an embedded metal fragment (HMTA) known to cause aggressive rhabdomyosarcomas in rats

Sensitivity was determined from the proportion of disease positive rats that test positive. Specificity was determined from the proportion of disease negative rats that test negative. Histopathology was the gold standard. Due to the aggressive nature of the disease process associated with the HMTA implants, it was not possible for the investigators to differentiate between the chronic inflammation and tumor formation processes. Therefore the sensitivity and specificity of using 18F-FDG PET-CT imaging actually combined both specific aims.

A decision matrix described by Park, Goo and Jo (2004) with a scale from one to five was used to classify disease status based on SUX max value (1= definitely benign, 2 = probably benign, 3= possibly malignant, 4= probably malignant, 5 = definitely malignant). A Receiver Operator Characteristic (ROC) analysis determined the ideal SUVmax cut off value of two. All SUVmax values less than or equal to two were benign and all values greater than two were malignant. PET–CT imaging had a sensitivity of 86%, specificity of 100% (Table 4).

Table 4: *Sensitivity and Specificity of ^{18}F-FDG SUVmax Each Time Point*

		Shape		
Week1		Sensitivity	Specificity	False Positive Rate
TP (1/8)	TN (8/8)	0.13	1.00	0
FP (0/8)	FN(7/8)			
Week 7				
TP (3/8)	TN (8/8)	0.38	1.00	0
FP (0/8)	FN(5/8)			
Week10				
TP (4/8)	TN (8/8)	0.50	1.00	0
FP (0/8)	FN(4/8)			
Week 13				
TP (4/8)	TN (8/8)	0.50	1.00	0
FP (0/8)	FN(4/8)			
Week 16				
TP (6/7)	TN (7/7)	0.86	1.00	0
FP (0/7)	FN(1/7)			

Note. TP – true positive; TN – true negative; FP – false positive; FN – False negatives

The area under the curve (AUC) was 0.938 . There were no false positives and at 16 weeks post implantation 14% of the disease positive rats classified as benign.

4. To sacrifice rats at each time point after x-ray and ^{18}F-FDG PET-CT to detect early signs of tumor development by histopathology.

Liver, kidney, spleen and testes weights were very similar between the TA and HMTA animal groups over all five of the time points. Sham control organ weights in week 16 were also very close to those of the other groups. An independent t- test was performed to A comparison of HMTA and Ta animal organ weights identified no significant differences (Table 5).

Table 5: *Organ Weight T-Test Comparison for Ta and HMTA Groups (N = 15)*

	Shape				
Organ	Group	Mean ± SEM	*t* (*df*)	*p*	*r*
Liver	Ta	37.43 ± 1.0	-.126 (28)	.901	.02
	HMTA	37.67 ± 1.6			
Kidney	Ta	5.86 ± .13	-.114 (28)	.910	.02
	HMTA	5.88 ± .15			
Spleen	Ta	2.06 ± .05	.567 (28)	.575	.01
	HMTA	2.02 ± .03			
Testes	Ta	8.66 ± .32	.448 (28)	.657	.08
	HMTA	8.43 ± .39			

Note. Organ weight (mg) to body weight (g) ratio (mg/g) used; *t*- t-test statistic; *df* –degrees of freedom; SEM – standard error of the mean; *p* – significance, significance is $p < .05$ (two tailed); *r* – effect size (.10 small, .30 medium, .50 large); Ta – Tantalum implanted animal; HMTA – Heavy Metal Tungsten Alloy implanted animal.

TA and HMTA animal groups had no significant differences in muscle to body weight ratios for the soleus, plantaris and gastrocnemius muscles when compared using a t-test (Table 6).

Table 6:
Muscle Weight T-Test Comparison for Ta and HMTA Groups (N = 15)

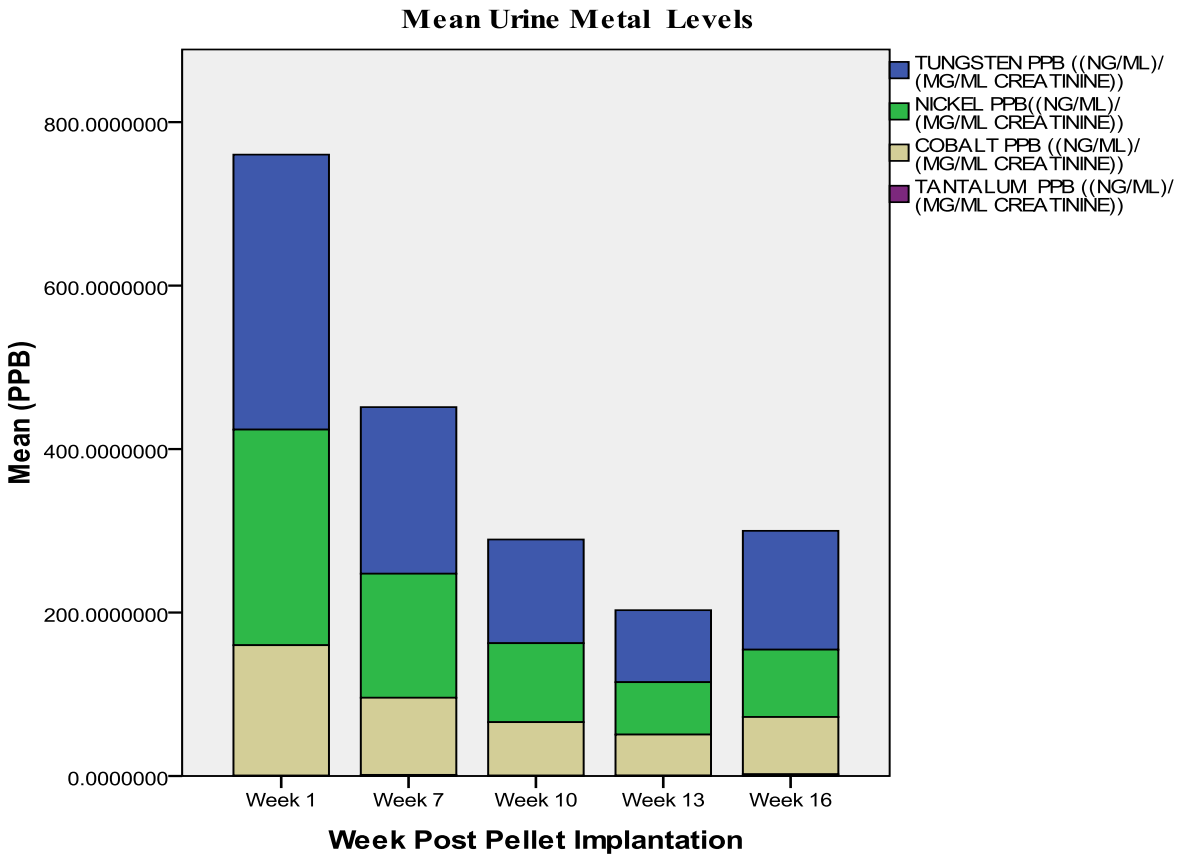
Muscle	Group	Mean ± SEM	*t* (*df*)	*p*	*r*
Soleus	Ta	.35 ± .01	-.096 (28)	.924	.02
	HMTA	.35 ± .01			
Plantaris	Ta	.88 ± .02	-.892 (28)	.380	.05
	HMTA	.91 ± .02			
Gastrocnemius	Ta	4.66 ± .06	.019 (28)	.985	.004
	HMTA	4.66 ± .07			

Note. Muscle weight (mg) to body weight (g) ratio (mg/g) used; *t*- t-test statistic; *df* –degrees of freedom; SEM – standard error of the mean; *p* –significance, significance is $p < .05$ (two tailed); *r* – effect size (.10 small, .30 medium, .50 large); Ta – Tantalum implanted animal; HMTA – Heavy Metal Tungsten Alloy implanted animal.

Animals implanted with the HMTA pellets (n=15) did not have visible or palpable tumors at sixteen weeks post pellet implantation. However, they all had histological signs of an invasive disease process. Typical skeletal muscle fibers are polygonal in shape, multinucleated with the nuclei on the periphery of the fiber when looking at a cross section. It is abnormal for skeletal muscle fiber nuclei to be centrally or internally located within the fiber (Karpati, Hilton-Jones, Busby, & Griggs, 2010). Large nuclei indicate active protein synthesis and odd or irregularly shaped nuclei are common in malignant tumors (Gisselsson, et al., 2001; Majno, & Joris, 2004; Webster, Witkin, & Cohen-Fix, 2009). Several atypical features observed in HMTA tissue sections such as central nuclei, increased number of nuclei, irregularly shaped nuclei, clusters of cells, rounded muscle fibers, and some muscle fibers without nuclei. Large numbers of nuclei and other cells infiltrated and destroyed muscle fibers in the area of the pellets. This 100% malignancy rate is consistent with the findings of Kalinich, et al. (2005). HMTA tissue specimen also stained positive for desmin over expression as early as thirteen weeks post implantation and stained positive for myoD1 as early as seven weeks post implantation. These antibodies along with other tests commonly used in the identification of rhabdomyosarcoma. As expected, the Ta animals (n=15) did not develop signs malignancy near the pellet sites during the study.

The most surprising finding was the invasive muscle fiber damage that was visible on histology slides as early as the first week post pellet implantation for the HMTA animals. HMTA implanted animals had not previously been sacrificed for histopathology this early after pellet implantation. Co, Ni, and W urine metal levels were highest in HMTA animals one week post implantation and then decreased until week sixteen post implantation (Figure 1). This means that the metals released into the tissues very quickly. Ta urine metal levels peaked at seven and sixteen weeks post implantation for both animal groups. These results are consistent with the urine metal findings of Kalinich, Vergara and Emond (2008).

Figure 1.



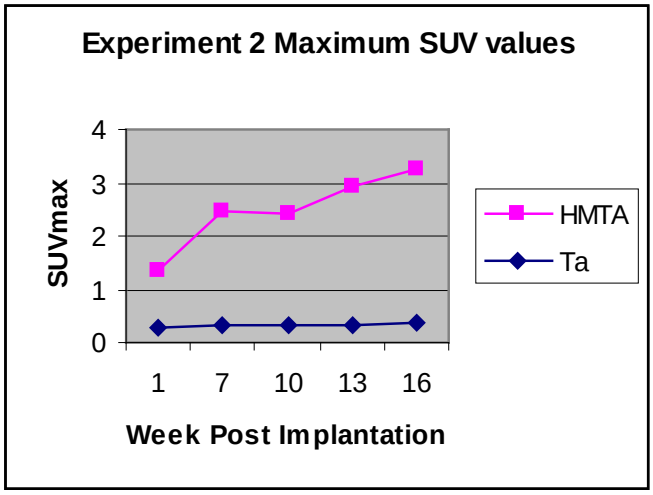
5. To compare phased radiograph (x-ray) images with PET-CT images for changes around implants over time between and within treatment groups.

Several findings were generated addressing Aim 5, first, x-rays provided very limited information. Metal pellet movement within the legs of several animals was detectable by the progression of five x-rays but there were no other observable pellet changes over the sixteen weeks. Shrapnel is known to migrate within soft tissues (Urban, Tomlinson, Hall, & Jacobs, 2004; Schroeder, Lowe, Chaimsky, Liebergall, & Mosheiff, 2010). Pellet movement added an uncontrollable element of difficulty to the pellet perimeter measurements. A second finding was that there was no significant difference in the pellet perimeter measurements between the HMTA and TA groups. However, there were a few statistically significant changes in pellet perimeter measurements between time points within both Ta and HMTA animal groups. This was probably due to measurement inaccuracies. The actual pellet dimensions are only 1mm x 2mm in size and there was over a 3mm difference in the median of the perimeter measurements for multiple time

points. Data from another much larger study that implanted the same type and size of Ta and HMTA pellets indicated no change in the pellet weights and shapes (Kalinich, et al., 2005).

A third important finding related to the information provided by PET-CT. PET-CT imaging captured the location of the metal pellets and changes in ^{18}F-FDG uptake in the region of interest (ROI) around the pellets over the sixteen weeks. ^{18}F-FDG uptake was significantly different between the Ta and HMTA animal groups. The Ta group maintained consistently low tracer uptake .The Kolmogorov-Smirnov Z test determined differences in SUVmax values for the Ta and HMTA animals. There was a significant difference between the Ta and HMTA SUVmax values ($Z = 1.73$, $p = .005$). Friedman's ANOVA determined differences in SUXmax values within the same groups over the sixteen weeks. There was no significant change in SUVmax values for the Ta animals over the sixteen weeks (X^2 (4) = 7.07, $p = .132$). A significant change in the SUVmax values for the HMTA animals occurred over the sixteen weeks (X^2 (4) = 15.07, $p = .005$). Post hoc follow up was a Wilcoxon signed-rank test. SUVmax values for the HMTA were significantly higher in week sixteen (Mdn = 2.77) than in week one (Mdn = 1.51), T = 6, $p = .028$, $r = -.64$. Illustrated below are the mean group differences in SUVmax values (Figure 2).

Figure 2:
Mean Maximum Standardized Uptake Value (SUV) for Tantalum (Ta) and Heavy Metal Tungsten Alloy (HMTA) Groups



The HMTA group had a significant within group change in tracer uptake from the first to the sixteenth week post pellet implantation ($p = .028$) and the effect of the change was large ($r = -.64$) (Cohen, 1988). PET-CT imaging did detect increased metabolic activity while x-rays gave no indication of the underlying neoplasia at any time over the sixteen

weeks. Thus, PET-CT imaging was beneficial in the early detection of cellular changes around the HMTA embedded fragments.

Relationship of current findings to previous findings:.

In this study, investigators observed metal pellet movement within the legs of several animals in the progression of five x-rays but there were no other observable pellet changes over the sixteen weeks. Shrapnel is known to migrate within soft tissues (Urban, Tomlinson, Hall, & Jacobs, 2004; Schroeder, Lowe, Chaimsky, Liebergall, & Mosheiff, 2010).
There was no significant difference in the pellet perimeter measurements between the HMTA and TA groups. Data from another much larger study that implanted the same type and size of Ta and HMTA pellets indicated no significant change in the pellet weights and shapes (Kalinich, et al., 2005).

While animals implanted with the HMTA pellets (n=15) did not have visible or palpable tumors at sixteen weeks post pellet implantation, they all had histological signs of an invasive disease process. Typical skeletal muscle fibers are polygonal in shape, multinucleated with the nuclei on the periphery of the fiber when looking at a cross section. It is abnormal for skeletal muscle fiber nuclei to be centrally or internally located within the fiber (Karpati, Hilton-Jones, Busby, & Griggs, 2010). Large nuclei indicate active protein synthesis and odd or irregularly shaped nuclei are common in malignant tumors (Gisselsson, et al., 2001; Majno, & Joris, 2004; Webster, Witkin, & Cohen-Fix, 2009). In this study, observed atypical features were central nuclei, increased number of nuclei, irregularly shaped nuclei, clusters of cells rounded muscle fibers and some fibers without nuclei. Large numbers of nuclei and other cells infiltrated and destroyed muscle fibers in the area. This 100% malignancy rate is consistent with the findings of Kalinich, et al. (2005). However, previous studies did not sacrifice animals to examine tissue histology earlier than 30 days post pellet implantation.

Co, Ni, and W urine metal levels were highest in HMTA animals one week post implantation and then decreased until week sixteen post implantation. This means that the metals released into the tissues very quickly. Ta urine metal levels peaked at seven and sixteen weeks post implantation for both animal groups. These results are consistent with the urine metal findings of Kalinich, Vergara and Emond (2008).

While the literature provided no previous studies using small animal PET-CT imaging to detect early tissue changes around embedded metals, PET-CT SUV max findings from this study were consistent with the results from previous small animal studies showing increased FDG uptake with carcinogenesis. Nanni and colleagues (2003) explored the accuracy of early detection of malignant cells with 18F-FDG PET. Human alveolar rhabdomysarcoma (RMS) xenografts (RH-30 cell line) injected in female nude mice. Sixteen of the twenty-three injected mice developed tumors. Each mouse was scanned four times. Ten of the mice had positive PET scans. Results showed the PET scans had a 90% (9/10) positive predictive value and a 46% (6/13) negative predictive value. Dewit and colleagues (2004) investigated the sensitivity and specificity of 18F-FDG PET imaging in the detection of recurrent RMS in fractionated radiotherapy treated R1H rat. ^{18}F-FDG PET was determined to be reliable in detecting tumor recurrence. Sensitivity increased with time but specificity did not. Raabe, Buchert, Seegers and de Wit (2006) used 18F-FDG PET to assess the recurrence of RMS (R1H-tumors) in a rodent

model. After a six months study, investigators concluded that 18F-FDG PET was beneficial in detection of reoccurrence of RMS following radio therapy as well as offering a time benefit of 26 to 67 days.

Effect of problems or obstacles on the results:

A major obstacle was the high demand for small animal PET-CT imaging at the CNRM translational imaging facility (TIF). There were multiple investigators with protocols requiring this technology. Due to the high volume of PET-CT scan requests, I worked with associate investigators and the TIF director and we reduced the number of PET-CT scans for this experiment. We have managed to decrease the required PET-CT scans by approximately half while still maintaining the specific aims and most of the original design.

One animal died in week one shortly after tracer injection for the PET-CT scan. Immediately notified, the PI replaced the deceased animal with an implanted back-up animal. The only real difference was that the replacement animal was not fasted six hours prior to the scan like all of the other animals. This was a Ta animal and did not appear impact the results.

Another obstacle was with the histopathology. The computer component of the Nikon imaging system (used to take histology slide pictures, muscle fiber measurements and counting) stopped working during data collection. Dr. Kasper was able to have loaner equipment delivered by the Nikon representative and data collection was completed.

Limitations:

1) Only a small number of animals were available for histological comparison at each time point (2 Ta, 2 HMTA) until week 16.

2) The pellet perimeter measurements were drawn free hand by the same investigator which could produce measurement error. The pellets moved, and the animals grew over the 16 weeks so the measurements were subjective from time point to time point.

3) Snap freezing the muscle tissue in isopentane cooled with liquid nitrogen and placed on dry ice until transferred to the -80 freezer resulted in freeze damage to some of the specimen. Formalin-fixed, paraffin-embedded tissue can give a much clearer picture, with better resolution. However, this procedure is not recommended for skeletal muscle. Skeletal muscle histology should be performed on frozen tissue (Karpati, Hilton-Jones, Bushby, & Griggs, 2010).

4) PET-CT imaging is expensive. The cost of imaging increased by 50% of original price quoted when the protocol was developed and the grant for funding submitted. In an effort to keep the protocol financially feasible, a few decisions were made at the start (e.g., number of animals) and modifications were made as needed (e.g., the sham control

animals were not scanned). Future studies may be able to use these pilot findings to improve the design of studies.

5) A last limitation relates to the translation of findings from animal studies to humans. Small animal PET-CT technology is comparable to large PET-CT scanners used on humans in medicine and clinical research and can provide a bridge to translate imaging experiments across species. However, the findings from this animal study cannot be directly extrapolated to humans. Rats are more sensitive to foreign body and radiation induced sarcomas than humans (Brand, Johnson, & Buoen, 1976; Hahn, Guilmette, & Hoover, 2002; McGregor, Baan, Partensky, Rice, & Wilbourn, 2000).

Conclusion:

Over the 16 week study period, the complete blood count and ELISA for C reactive protein provided no evidence of a chronic inflammatory changes over time. The aggressive cellular response of implanted rats to the HMTA made it impossible to differentiate the sensitivity and specificity of FDG as a biomarker of chronic inflammation from tumor formation. However, the sensitivity of the PET-CT imaging for the HMTA animals increased over the five time points and there were no false positives. Histopathology examination as the gold standard showed that increased FDG uptake was associated with an aggressive malignancy in nearly all of the HMTA implanted animals . PET–CT imaging had a sensitivity of 86% and specificity of 100%. Another sign of metal deterioration found was the detection of metal in the urine. X-ray radiographs provided minimal information on implanted metal changes over the 16 week time-period. PET-CT imaging provided early detection of metabolic changes occurring at the site of the implanted HMTA. Since clinical practice conflicts over the removal all metal fragments, diligent surveillance is crucial. PET-CT imaging should be considered as a possible tool. More importantly, clinicians must be aware that embedded metals may deteriorate and move over time, potentially leading to pathological responses.

Significance of Study or Project Results to Military Nursing

Many of the over 50,000 service members wounded in the Global War on Terror are living with embedded metal fragments from war in their bodies. Current literature on tissues changes occurring around retained fragments is limited; thus, the long-term health implications of exposure to embedded metal fragments are a concern. Current surveillance programs use traditional two-dimensional X-ray imaging to detect changes in the metal fragments. X-ray does not capture soft tissue images or cellular and metabolic activity. X-ray does provide clinicians with information about molecular changes that may signal malignant or pathological changes.

This pilot study found that small animal positron emission tomography-computed tomography (PET-CT) has the sensitivity to detect neoplastic changes in muscle tissue of a Fischer 344 rats exposed to an embedded tungsten alloy. Increased FDG uptake in the area of the metal indicated early neoplastic changes before any change in the metal appeared on x-ray. This study provides new knowledge on the use of small animal PET-CT imaging as a surveillance tool for monitoring embedded fragments. While the findings of this study cannot be generalized to humans, it offers hope that with additional research PET-CT imaging might serve as a tool in the surveillance of humans with embedded fragments.

The findings from this study indicate that more work needs to be done to determine if embedded fragments are harmful for humans. ^{18}F-FDG is a nonspecific and exhibits increased uptake in areas of inflammation, infection, in addition to other areas of increased glucose metabolism including those resulting from the formation of some tumors (Love, Thomas, Tronco, & Palestro, 2005). Inflammation is a component of malignant tumors and also some benign tumors (Majno & Joris, 2004). This can potentially lead to false positive results in patients with healing surgical wounds or other injuries in addition to a tumor (Brown, et al., 2012). Future research should include animal studies to determine how different shrapnel metals effect wound healing and how this wound healing effects 18F-FDG uptake. Studies with human subjects are necessary. The current embedded fragment surveillance system already could include PET-CT imaging as part of their protocol. A pilot study using PET-CT on a random sample of veterans and service members in the toxic embedded surveillance center or a large military medical facility with controls could provide valuable information. Participants would have positive urine metal levels for at least one metal from a list of toxic metals currently monitored by the Department of Defense. The sensitivity of PET-CT to detect metabolic changes around the embedded fragments would be evaluated. Examine the barriers to having removed embedded fragments analyzed for composition since IEDS are composed of a variety of materials and thus have different health implications. Genetic testing is another consideration that should be considered within the population of wound warriors with embedded fragments. Studies with *in vitro* testing of W, Ni, Co and several other metals used in munitions were found to be genotoxic and mutagenic (Miller, Xu, Stewart, Emond et al., 2000; Miller, Mog et al., 2001; Miller, Xu, Stewart, Prasanna & Page, 2002; Miller, Brooks, Smith & Page, 2004). DNA testing is routine for most service members when entering the military thus a comparison of the initial DNA with a post combat injury samples over time may provide information on changes associated with retained toxic metal exposure.

As additional knowledge is gained in this area, there is great potential that it will influence how nurses care for patients with embedded shrapnel. Metals can be very reactive in the body. Patients with shrapnel could have changes in blood pressure, liver, kidney or

neurological function which could all be related to metal toxicity. Exploration of the health effects of exposure to embedded fragments is just beginning. As more is learned about how these fragments react in the body, ways to mitigate or eliminate any harmful effects can be developed. This work contributes additional knowledge and serves as a step to a larger study focused on the long term health effects of exposure to embedded metal fragments. There are also several areas where findings could be translated to practice in the military:

- Educate nurses and other health care providers to inquire about a patient history of combat injuries, retained shrapnel in their body
- When retained shrapnel is identified, monitor patients for fragment migration and tissue changes.
- Educate patients and providers of the possibility of early tissue changes around embedded fragments.
- Educate patients and providers on the proper handling of removed fragments (sent to lab for composition analysis)

Monitoring should include an evaluation of the fragment (s) after removal from a patient. Until an embedded fragment is removed and analyzed for composition, it is difficult to determine exactly what exposure occurred. Lastly, if the patient is not already on the Toxic Embedded Fragment Surveillance Center (TEFSC) registry, nurses can encourage them to consider it.

Changes in Clinical Practice, Leadership, Management, Education, Policy, and/or Military Doctrine that Resulted from Study or Project

None to date

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Summary of Dissemination

Carefully document all dissemination activities; delete unused categories and/or rows; add rows to the appropriate categories as needed

Type of Dissemination	Citation	Date and Source of Approval for Public Release
Publications (using a consistent reference style, provide complete citation for papers already published in print or electronic journals)		
Publications in Press (using a consistent reference style, provide partial citation for papers accepted for publication but not yet published in print or electronic journals; if known, provide estimated date paper will be published)		
Published Abstracts (using a consistent reference style, provide complete citation for abstracts published in print or electronic journals)		
Podium Presentations (using a consistent style, provide author(s), title of presentation, conference name, conference location, date of presentation, sponsoring agency or organization)		
Poster Presentations (using a consistent style, provide author(s), title of		

poster, conference name, conference location, date of presentation, sponsoring agency or organization)		
Media Reports (provide details such as title, type of media [e.g., press release, newspaper article, television or radio story, internet post], date of report)		
Other		

Reportable Outcomes

Carefully document reportable outcomes; add rows to the appropriate categories as needed

Reportable Outcome	Detailed Description
Applied for Patent (if none, type "none")	None
Issued a Patent (if none, type "none")	None
Developed a cell line (if none, type "none")	None
Developed a tissue or serum repository (if none, type "none")	None
Developed a data registry (if none, type "none")	None

Recruitment and Retention Aspect		Number
Animals Projected in Grant Application		36
Animals Purchased	(2 were no charge for a total of 36)	34
Model Development Animals		4
Research Animals		32
Animals With Complete Data		32
Animals with Incomplete Data		0

Recruitment and Retention Aspect		**Number**	
Animals Projected in Grant Application		36	
Animals Purchased	(2 were no charge for a total of 36)	34	
Model Development Animals		4	
Animals Intervention Group / Control or Sham Group		15	15+2
Intervention Group / Control or Sham Group Animals With Complete Data		15	15 +2
Intervention Group / Control or Sham Group Animals With Incomplete Data		0	0

Final Budget Report

Attach and sign the official final budget from your applicant organization. Your signature indicates that you carefully reviewed the official final budget and agree with it. If applicable, briefly describe the rationale for and outcome of reallocating funds and/or obtaining additional funds. If applicable, explain reason(s) for remaining funds.

There are a few main reasons why funds still remain in the account. First, the original price quote for PET-CT imaging was $100.00 per scan. This was prior to the start of fiscal year 2012. At the beginning of the new fiscal year the pricing changed and I was informed that the PET-CT imaging would cost $150.00 per scan/per hour. I originally planned and budgeted for 130 scans at 100.00 per scan and the increase would have substantially exceeded the funds of the grant. Next, due to the high demand for the small animal PET-CT imaging in the CNRM translational imaging facility (TIF), I was required to decrease the number of PET-CT scans in my protocol in order to maintain my timeline and still complete the protocol. This TSNRP approved modification was achieved through a collaborative effort with my mentors and the director of the TIF while addressing the original aims of the study. The total number of PET-CT scans decreased from the originally proposed 130 to 78. This decrease also the reduced the number of tracer doses that were purchased. Ultimately, because of the decrease in PET- CT scans the grant still provided sufficient funds to cover the expenses of the study with some funds remaining. Lastly, the $233 dollars that remained in the original budget was set aside for dissemination. I am currently working a manuscript for publication and will submit abstracts and a poster for conferences in 2013. Unfortunately, there is not enough time remaining to use the funds allocated for dissemination before the close of this grant. These are the reasons for the remaining funds.