Supplementary Analyses for: Comparison of Pro-Inflammatory Cytokines and Bone Metabolism Mediators Around Laser-Lok and Machined Transmucosal Abutments: A Pilot, Randomized Clinical Trial

Prepared for Christopher A. Barwacz, DDS, FAGD (PI) by
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Statistical Methods

Comparison of GCF to PICF

Log-cytokine values were compared between natural teeth and implants using the paired T-test and the Wilcoxon Signed Rank test. Descriptive statistics were prepared to aid interpretation.

P-values were adjusted for multiple comparisons using the Holm (1979)[1] method. All analyses were performed using R 3.4.2 (Vienna, Austria) and SAS 9.4 (Cary, NC) at the 5% level of significance.

[1] Holm, S. (1979). A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics, 6, 65–70. http://www.jstor.org/stable/4615733.

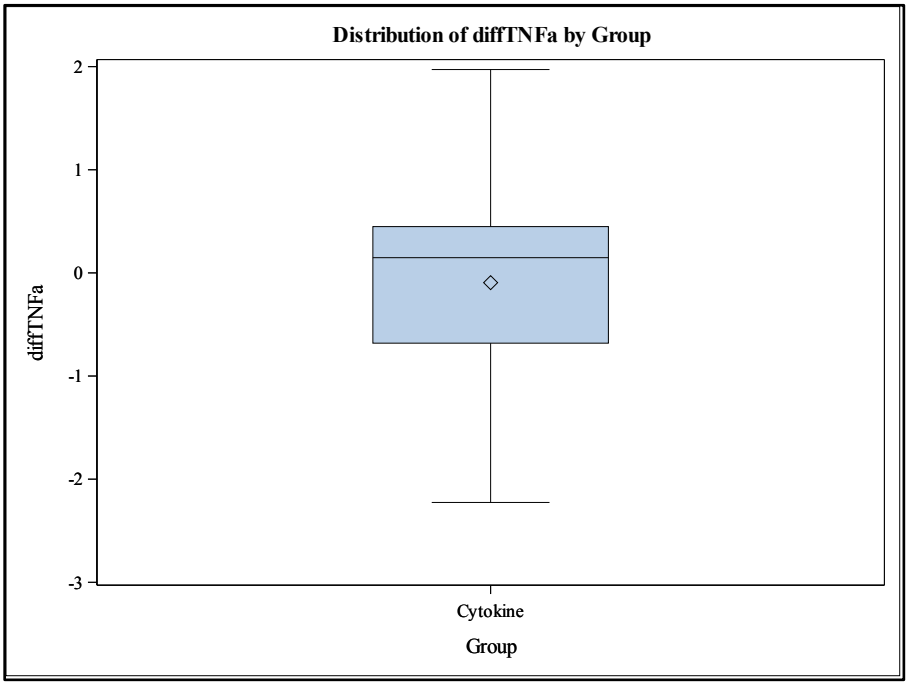
Aim 1: Do cytokine levels differ between GCF and PICF?

Descriptive Statistics

Table 1. Descriptive Statistics (PICF – GCF)

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
TNFa	12	-0.09	1.07	0.15	-2.23	1.97
IL6	12	-0.38	0.74	-0.21	-2.50	0.35
IL8	12	-0.27	1.32	-0.23	-2.52	1.91
MMP1	12	-0.11	0.76	-0.08	-1.22	1.25
IP10	12	-0.28	1.05	-0.25	-3.02	0.93
MMP8b	12	-0.14	0.71	-0.08	-1.18	0.99
IL_1B	12	0.17	0.92	0.06	-1.67	2.15
MMP13	12	0.34	1.70	0.34	-2.30	4.70
ENA78	12	-0.18	0.76	0.01	-1.84	0.70
MIG	12	-0.12	0.34	-0.03	-0.78	0.52
Osteopontin	12	-0.22	1.24	0.04	-2.47	1.76
ITAC1	12	0.19	1.03	0.26	-1.92	2.45
Osteoprotegerin	12	-0.12	1.18	-0.10	-2.53	2.72

Boxplots


Distribution of diffTNFa by Group
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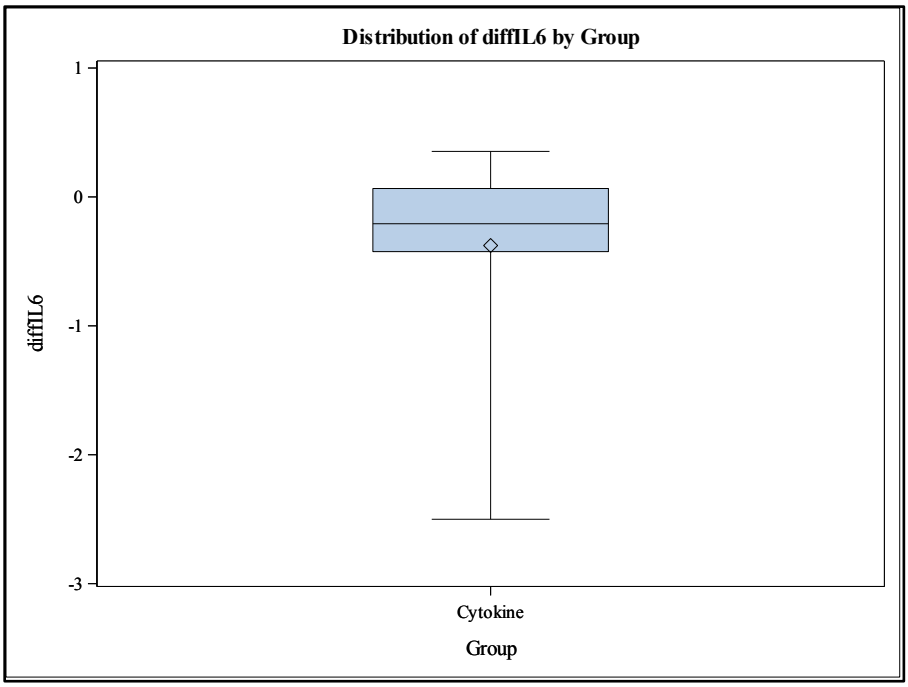
-3
diffTNFa
Cytokine
Group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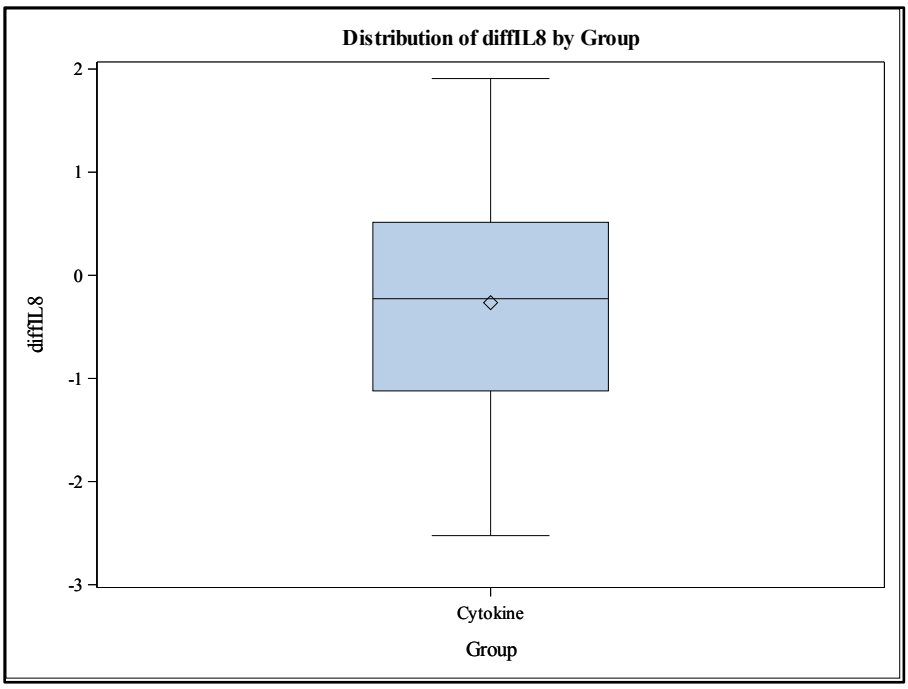
Cytokine
Group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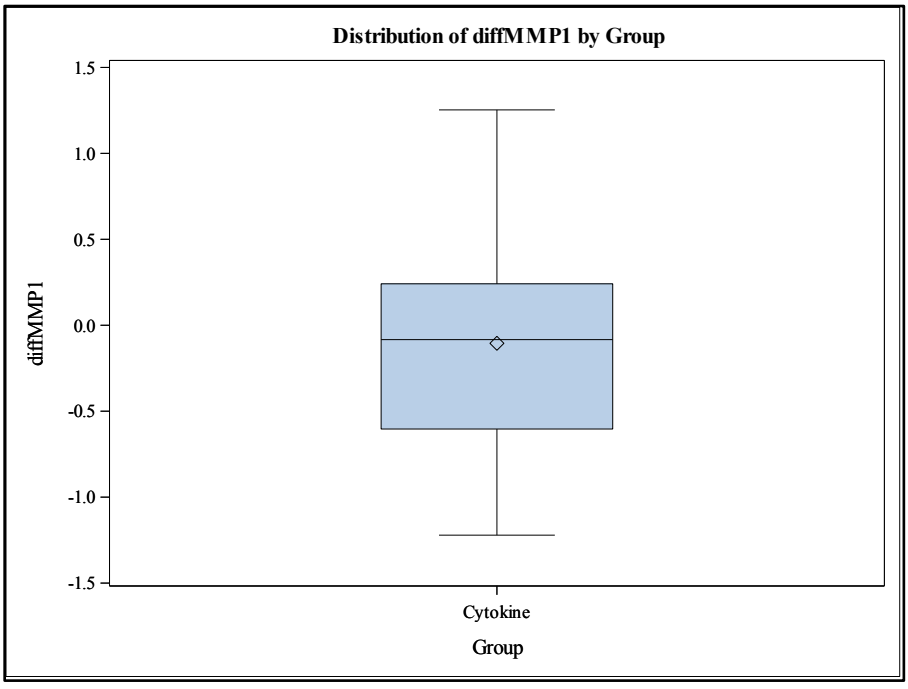
diffIL8
Cytokine
Group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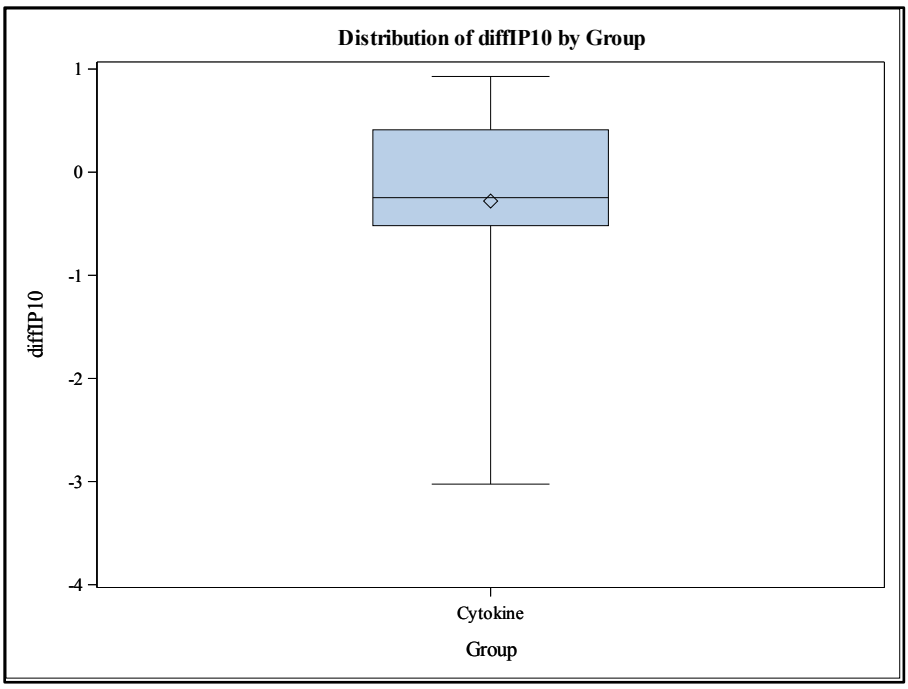
diffMMP1
Cytokine
Group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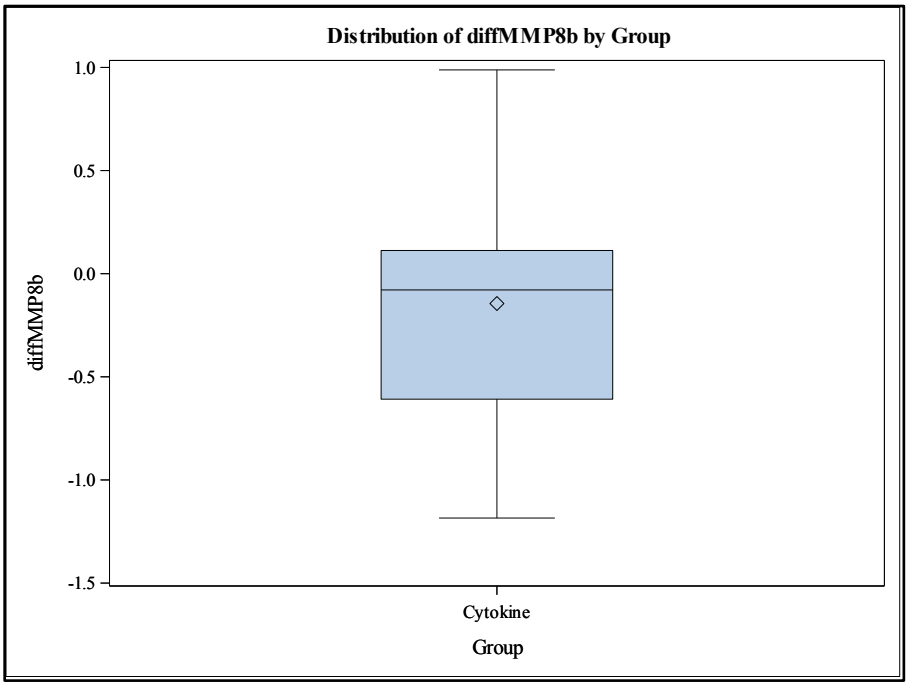
diffIP10
Cytokine
Group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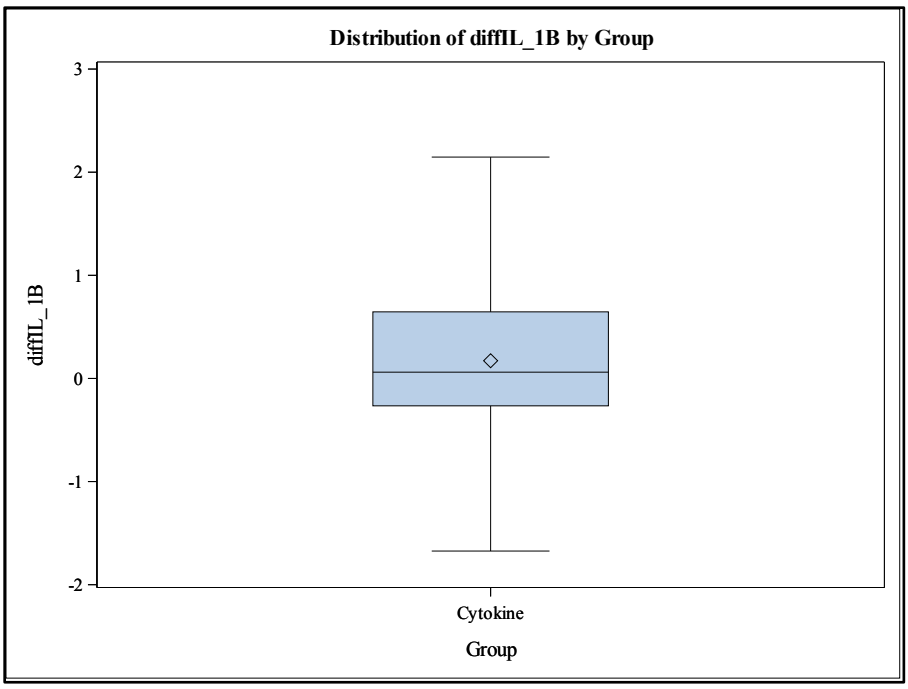
diffMMP8b
Cytokine
Group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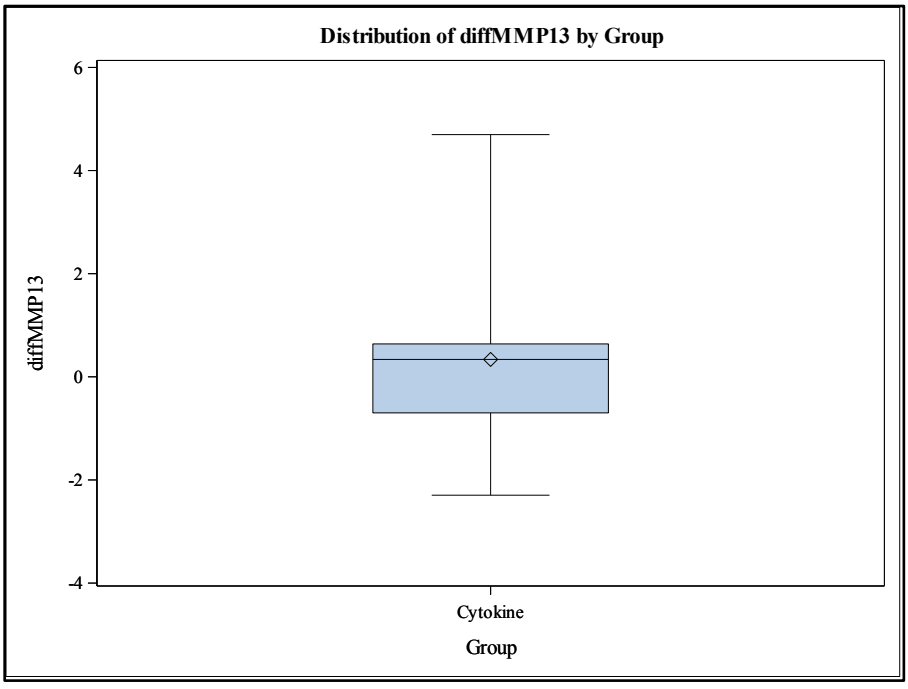
-1
-2
Cytokine
Group


Distribution of diffMMP13 by Group
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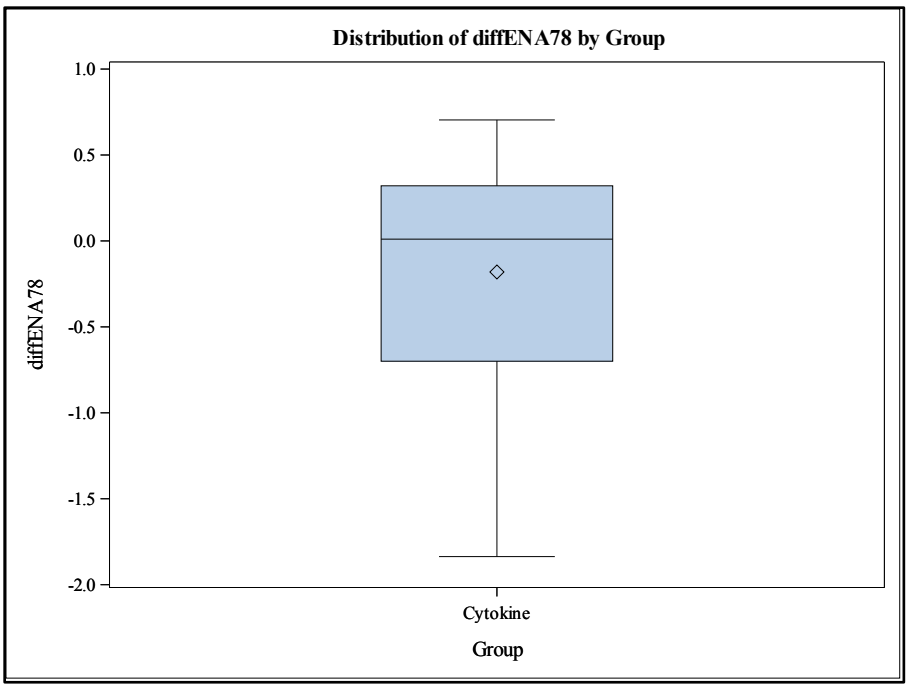
2
0
-2
-4
Cytokine
Group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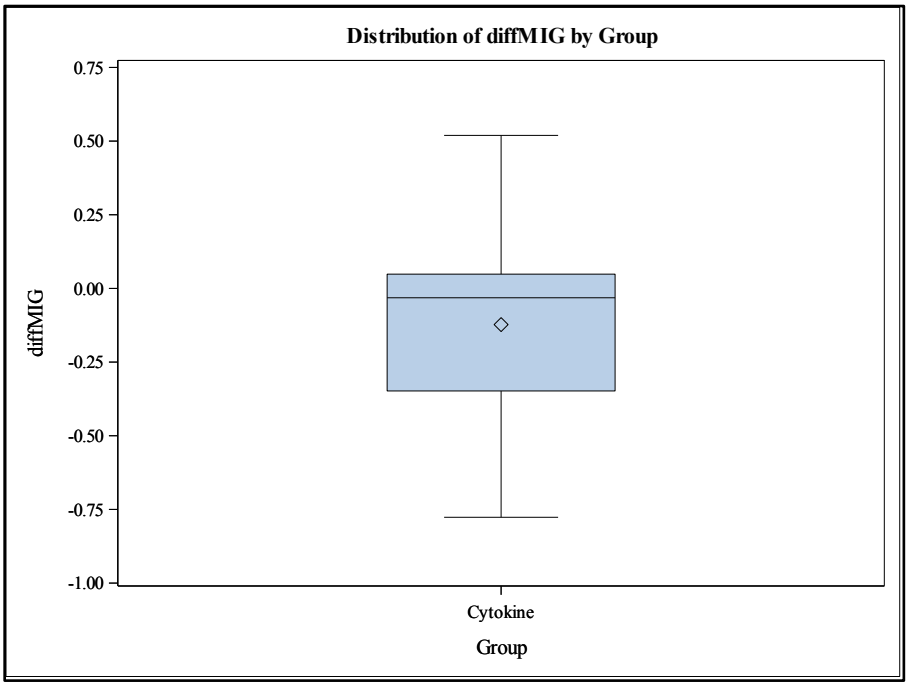
diffENA78
Cytokine
Group


Distribution of diffMIG by Group
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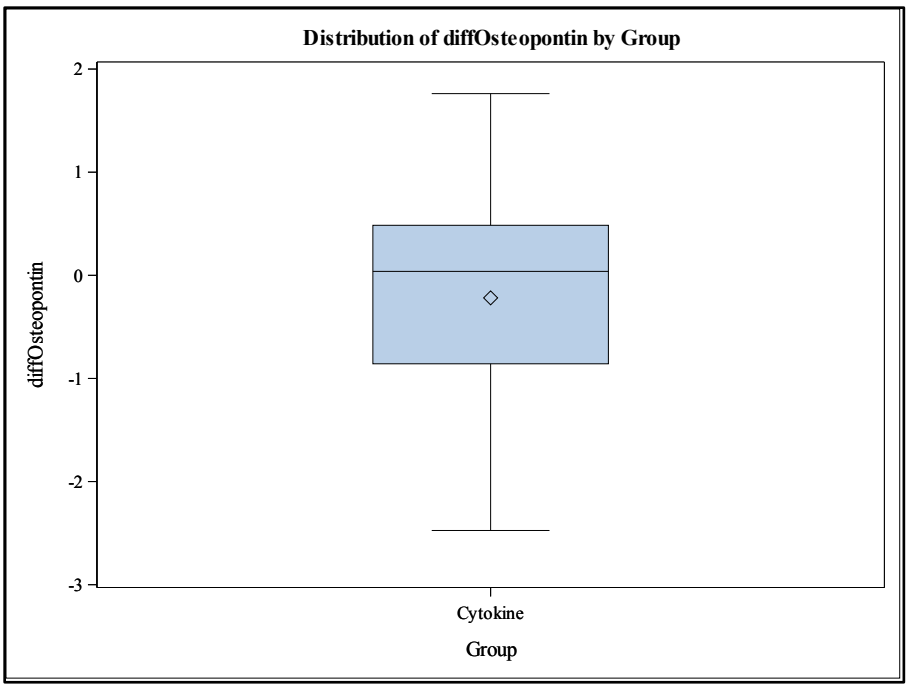
Cytokine
Group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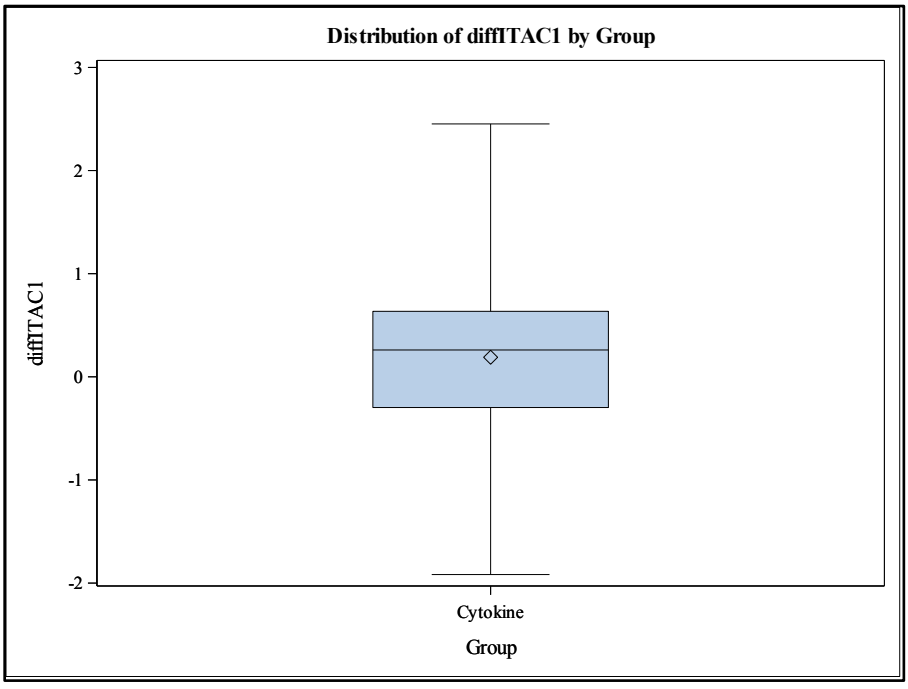
diffOsteopontin
Cytokine
Group


Distribution of diffITAC1 by Group
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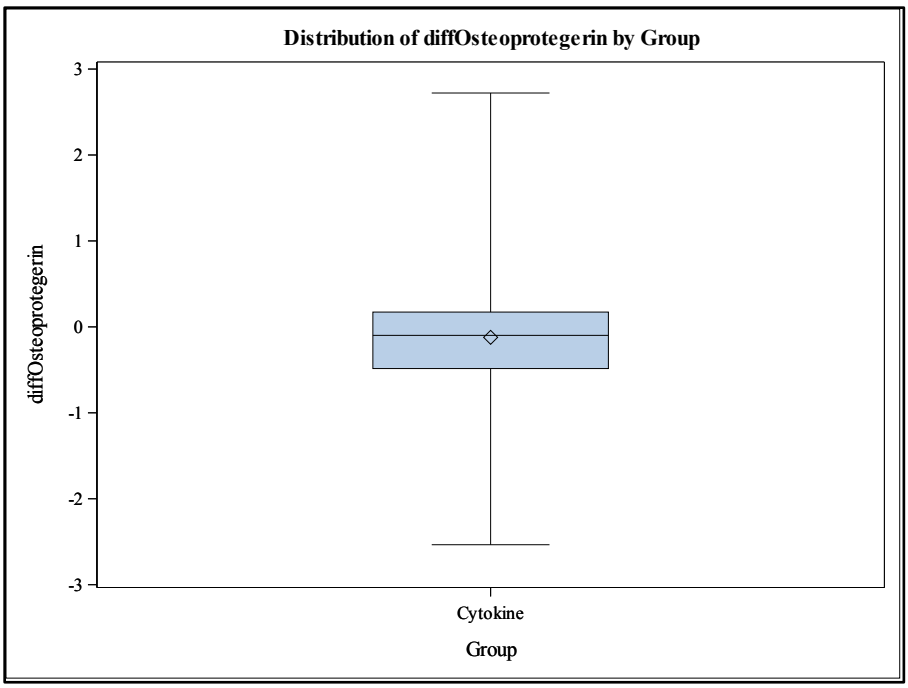
-1
-2
diffITAC1
Cytokine
Group


Distribution of diffOsteoprotegerin by Group
3
2
1
0
-1
-2
-3
diffOsteoprotegerin
Cytokine
Group

GCF and PICF Comparisons

Table 2. Two-Sample Tests Comparing PICF Cytokine Levels					
		T-test		Wilcoxon Signed Rank	
Measurement	N	P-value	Adjusted P-value	P-value	Adjusted p-value
TNFa	12	0.7648	0.7648	0.9097	0.9097
IL6	12	0.1063	0.7199	0.0425	0.5525
IL8	12	0.5011	0.7199	0.5186	0.9097
MMP1	12	0.6428	0.7596	0.7334	0.9097
IP10	12	0.3760	0.7199	0.5693	0.9097
MMP8b	12	0.4930	0.7199	0.3804	0.9097
IL-1B	12	0.5294	0.7199	0.6221	0.9097
MMP13	12	0.5060	0.7199	0.7334	0.9097
ENA78	12	0.4302	0.7199	0.7910	0.9097
MIG	12	0.2323	0.7199	0.3394	0.9097
Osteopontin	12	0.5538	0.7199	0.8501	0.9097
ITAC1	12	0.5357	0.7199	0.3911	0.9097
Osteoprotegerin	12	0.7268	0.7648	0.4697	0.9097

Conclusion for GF and PICF Comparisons

Before adjustment, the Wilcoxon Signed Rank test found that IL6 was higher in GCF (P = 0.0425).

After adjustment, no significant differences were detected.

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Contents

Statistical Methods

Comparison of PICF

Log-cytokine values were compared between the Laser-Lok and Machined implants using the two sample T-test and the Wilcoxon Rank Sum test. Descriptive statistics and box plots were prepared to aid interpretation.

Comparison of GCF

Log-cytokine values were compared between the groups of natural teeth using the two sample T-test and the Wilcoxon Rank Sum test. Descriptive statistics and box plots were prepared to aid interpretation.

Cluster Analyses

Exploratory cluster analyses were performed for subjects and cytokines using the implant values and the natural tooth values (separately). Hierarchical clustering with agglomeration via complete linkage was performed on dissimilarity measures based on Euclidean distances.

P-values were adjusted for multiple comparisons using the Holm (1979)[1] method. All analyses were performed using R 3.4.2 (Vienna, Austria) and SAS 9.4 (Cary, NC) at the 5% level of significance. Analyses were performed using the log-transformed values.

[1] Holm, S. (1979). A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics, 6, 65–70. http://www.jstor.org/stable/4615733.

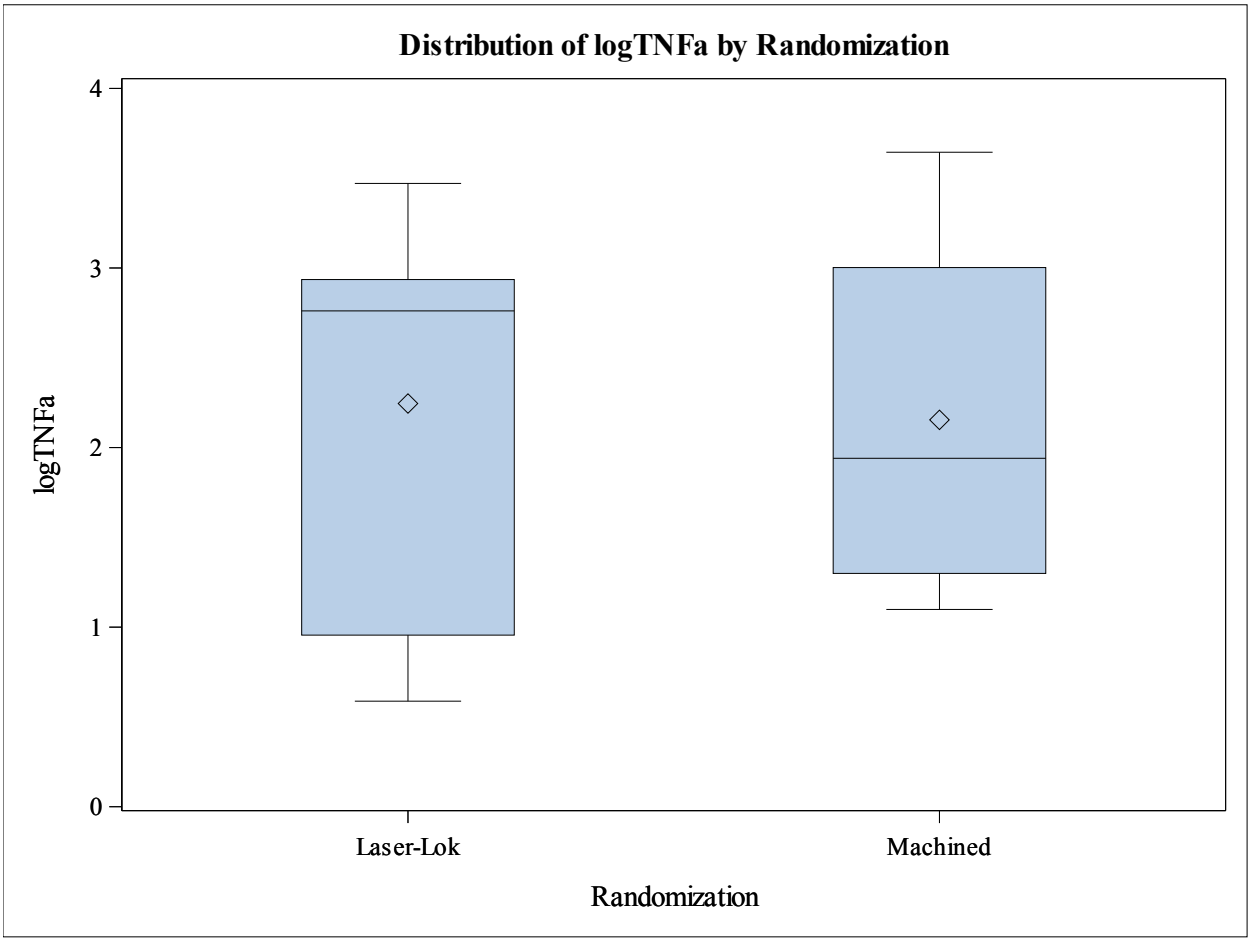
Aim 1: Do the cytokine levels of the Laser-Lok PICF differ from the Machined PICF?

Descriptive Statistics

Table 1. Descriptive Statistics for PICF Cytokine Levels

Implant	Variable	N	Mean	Std Dev	Median	Minimum	Maximum
Laser-Lok	logTNFa	6	2.25	1.18	2.76	0.59	3.47
	logIL6	6	1.80	0.31	1.84	1.38	2.12
	logIL8	6	6.54	0.60	6.54	5.69	7.32
	logMMP1	6	4.48	0.64	4.37	3.76	5.51
	logIP10	6	1.29	0.79	1.09	0.43	2.30
	logMMP8b	6	11.37	0.37	11.38	10.77	11.90
	logIL_1B	6	5.19	0.61	5.26	4.29	5.91
	logMMP13	6	5.73	0.94	5.52	4.60	7.14
	logENA78	6	4.05	0.53	3.97	3.52	4.97
	logMIG	6	6.95	0.12	6.98	6.76	7.07
	logOsteopontin	6	6.44	1.19	6.90	4.01	7.03
	logITAC1	6	2.53	0.36	2.35	2.21	3.07
	logOsteoprotegerin	6	2.94	0.95	2.68	1.93	4.45
Machined	logTNFa	6	2.15	1.00	1.94	1.10	3.64
	logIL6	6	1.61	0.36	1.71	0.94	1.92
	logIL8	6	5.52	1.06	5.74	3.75	6.48
	logMMP1	6	3.89	0.72	3.98	2.58	4.69
	logIP10	6	1.32	1.09	1.36	-0.41	2.90
	logMMP8b	6	10.61	1.11	10.58	8.84	11.88
	logIL_1B	6	4.92	1.08	4.57	3.74	6.66
	logMMP13	6	5.78	1.08	6.11	3.69	6.77
	logENA78	6	3.27	0.65	3.51	1.98	3.69
	logMIG	6	6.63	0.51	6.68	5.72	7.16
	logOsteopontin	6	6.50	0.96	6.54	5.30	7.67
	logITAC1	6	2.56	0.84	2.32	1.94	4.10
	logOsteoprotegerin	6	3.38	1.06	3.38	1.91	4.68

Box Plots


Distribution of logTNFa by Randomization
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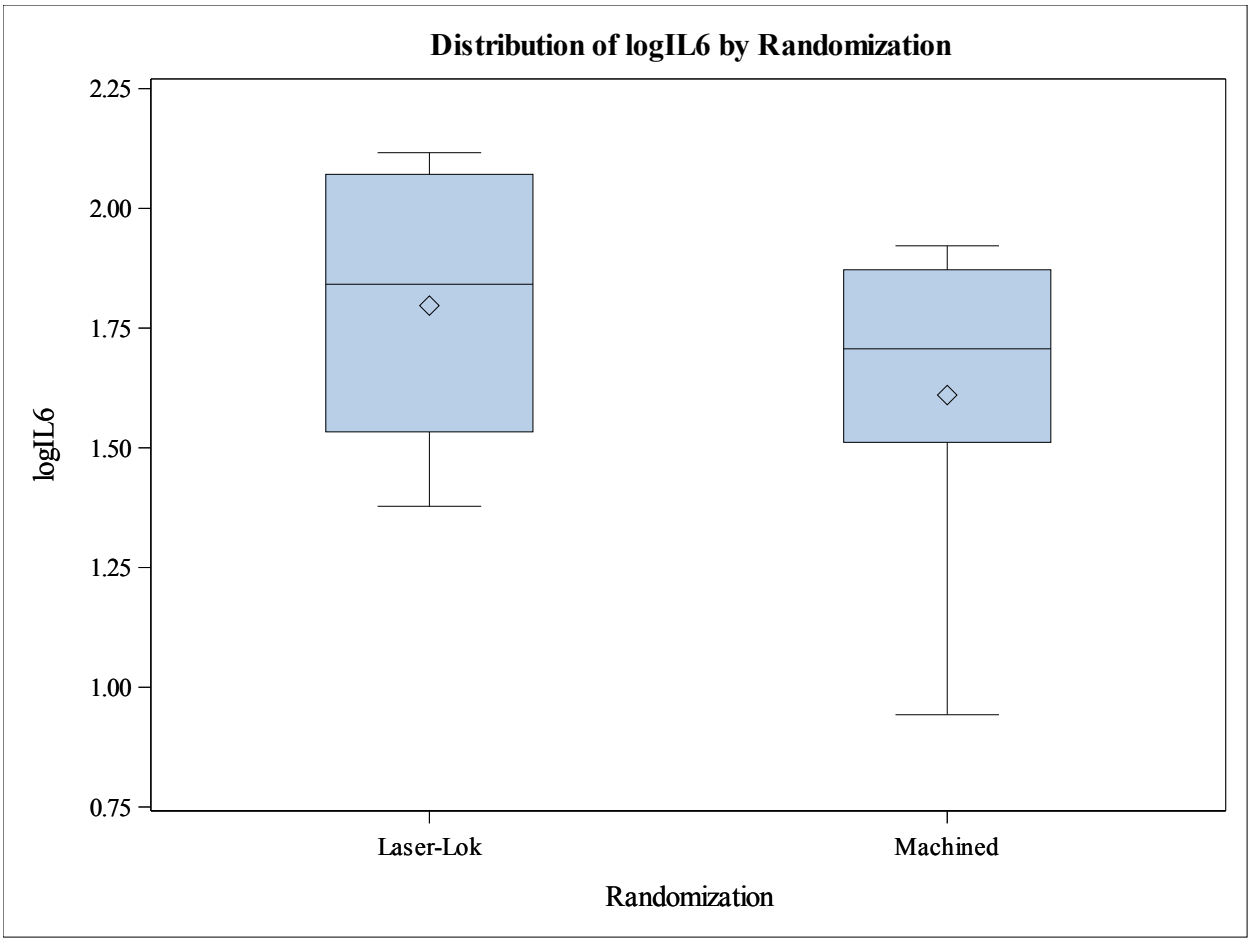
0
logTNFa
Laser-Lok
Machined
Randomization


Distribution of logIL6 by Randomization
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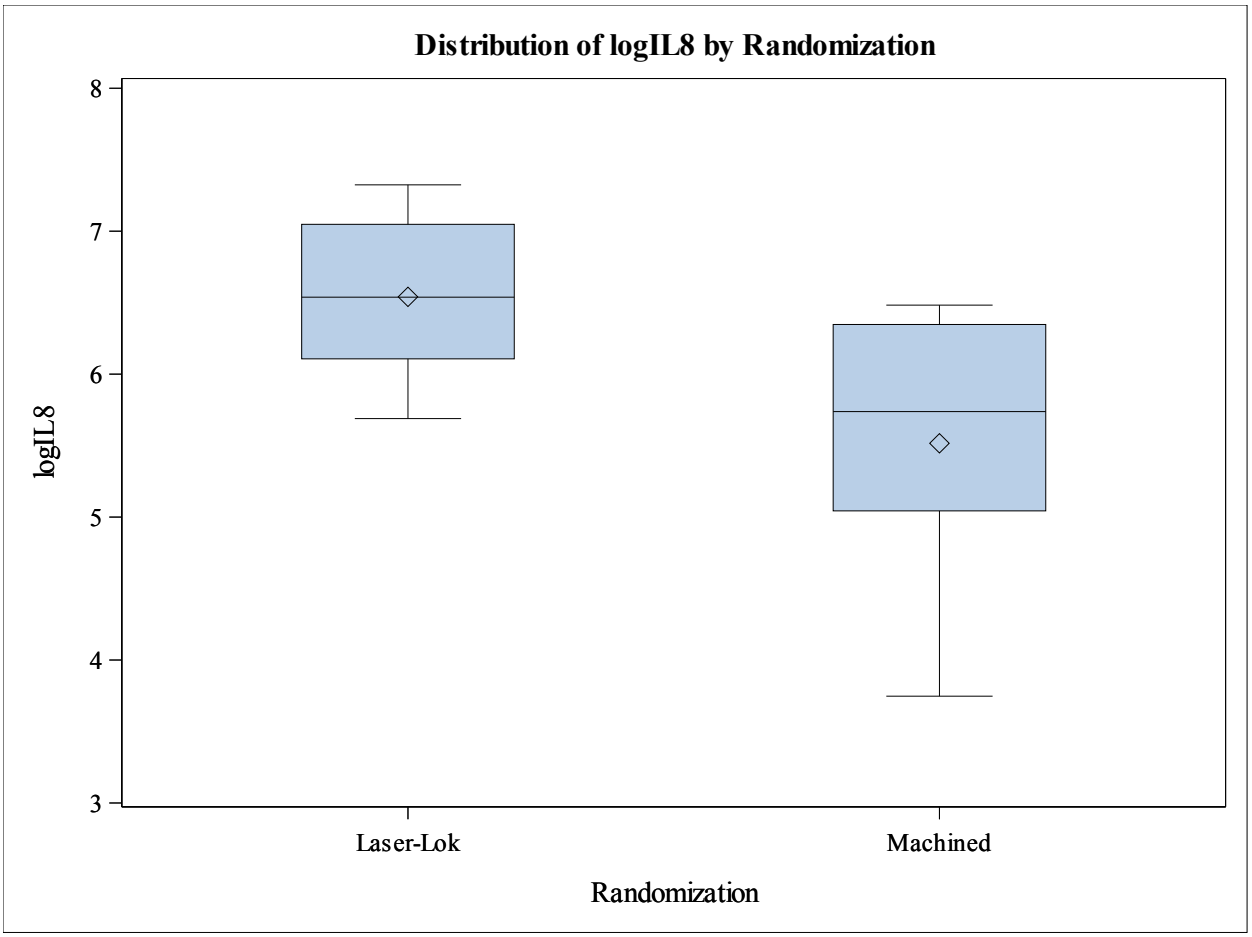
logIL6
Laser-Lok
Machined
Randomization


Distribution of logIL8 by Randomization
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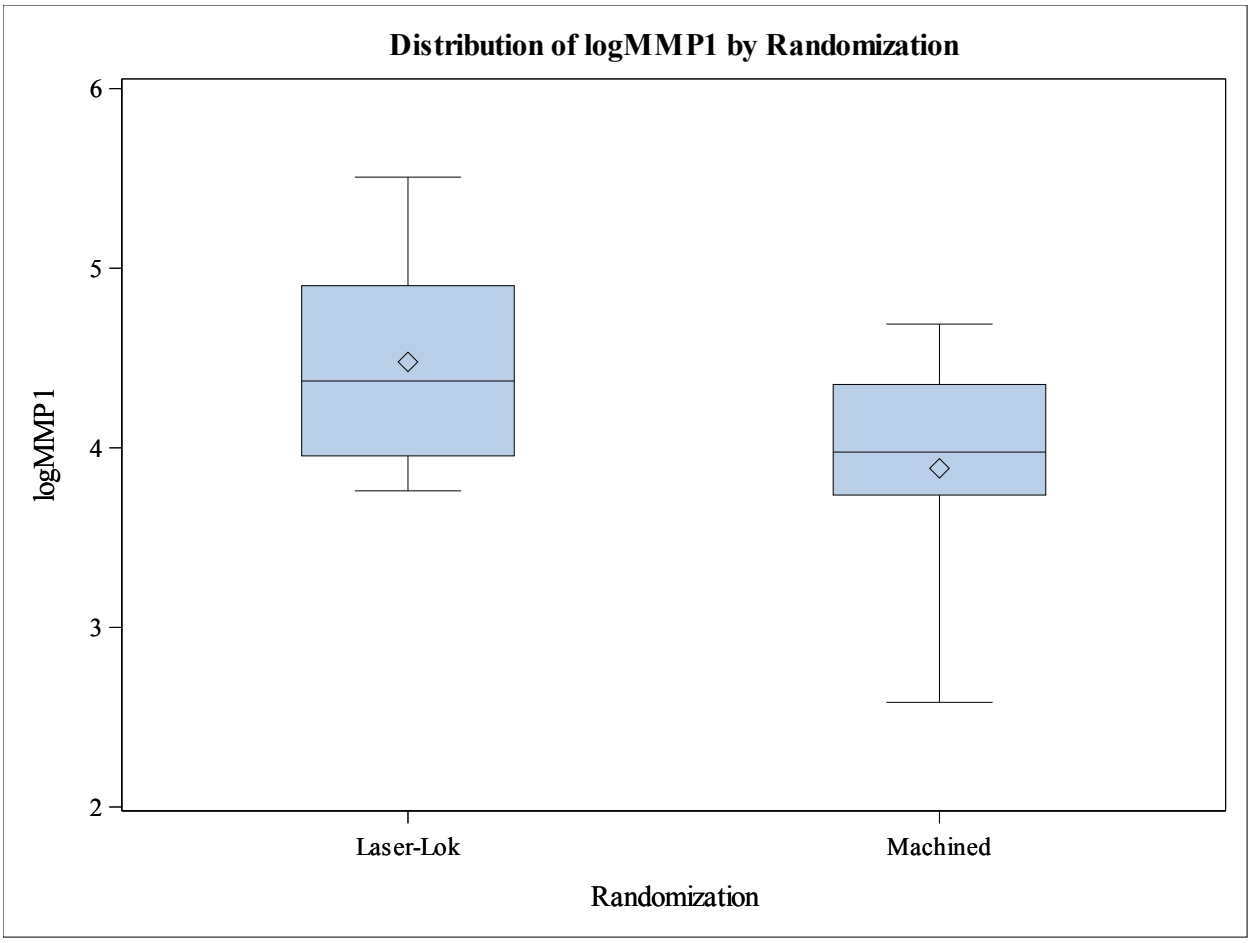
5
4
3
logIL8
Laser-Lok
Machined
Randomization


Distribution of logMMP1 by Randomization
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3
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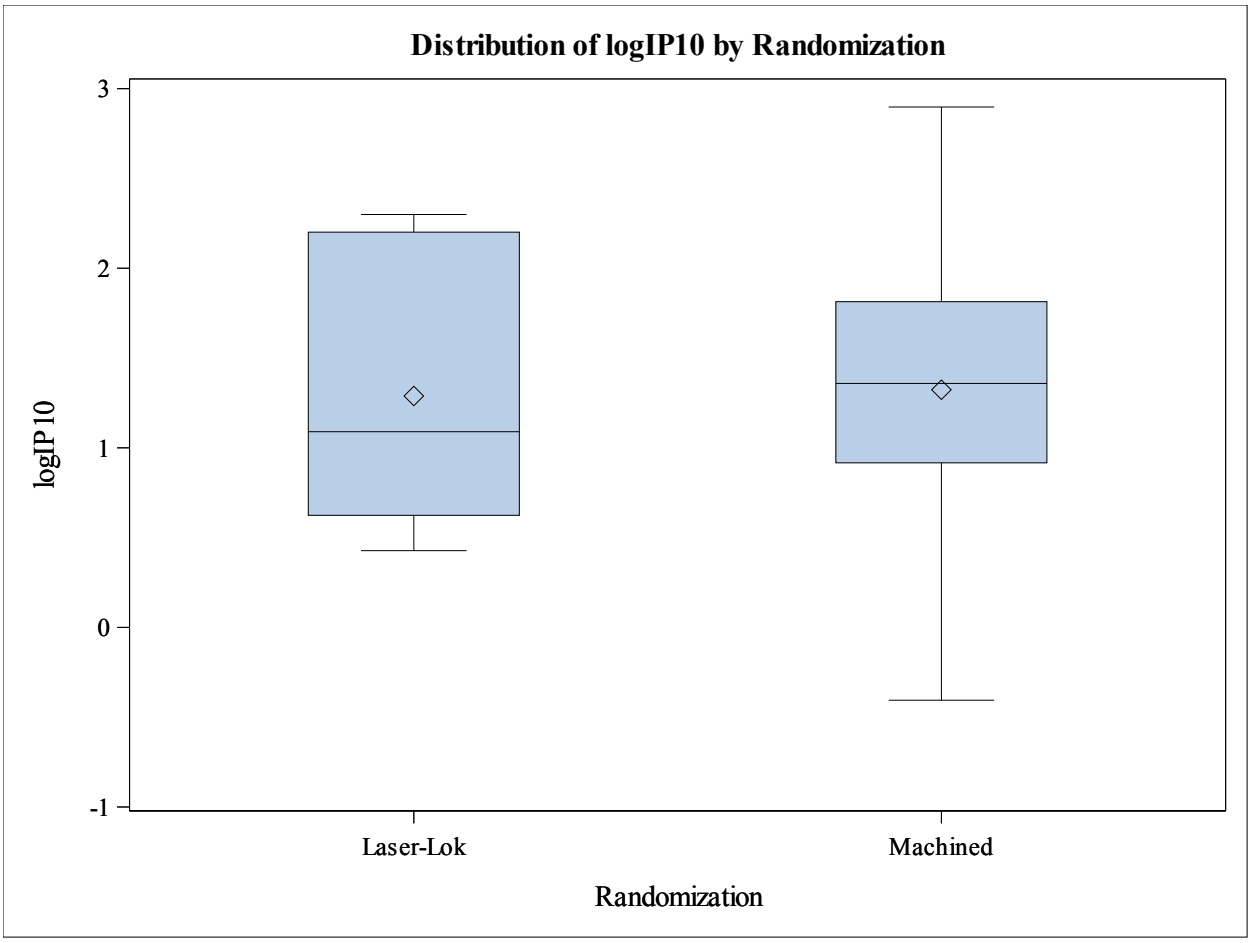
logMMP1
Laser-Lok
Machined
Randomization


Distribution of logIP10 by Randomization
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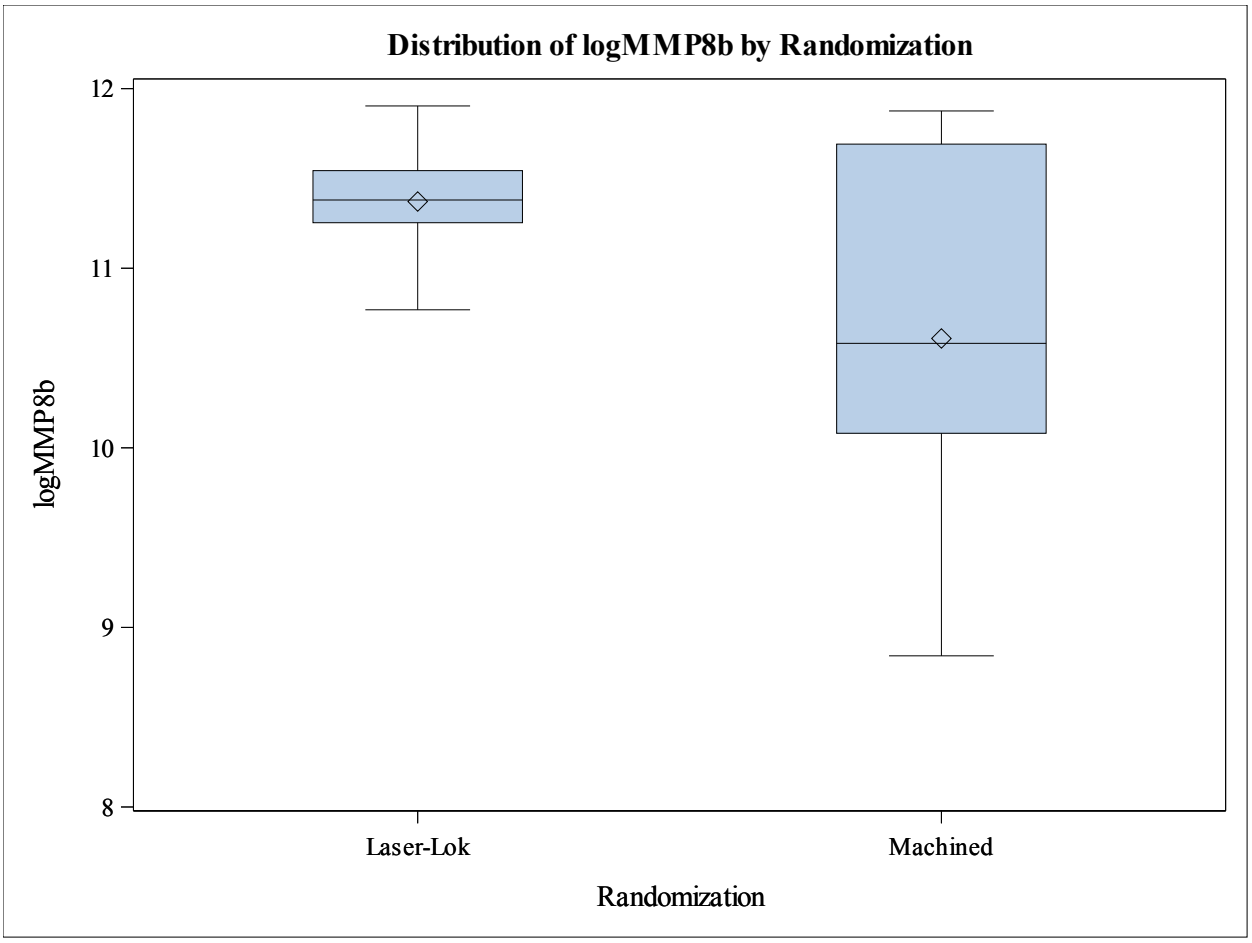
logIP10
Laser-Lok
Machined
Randomization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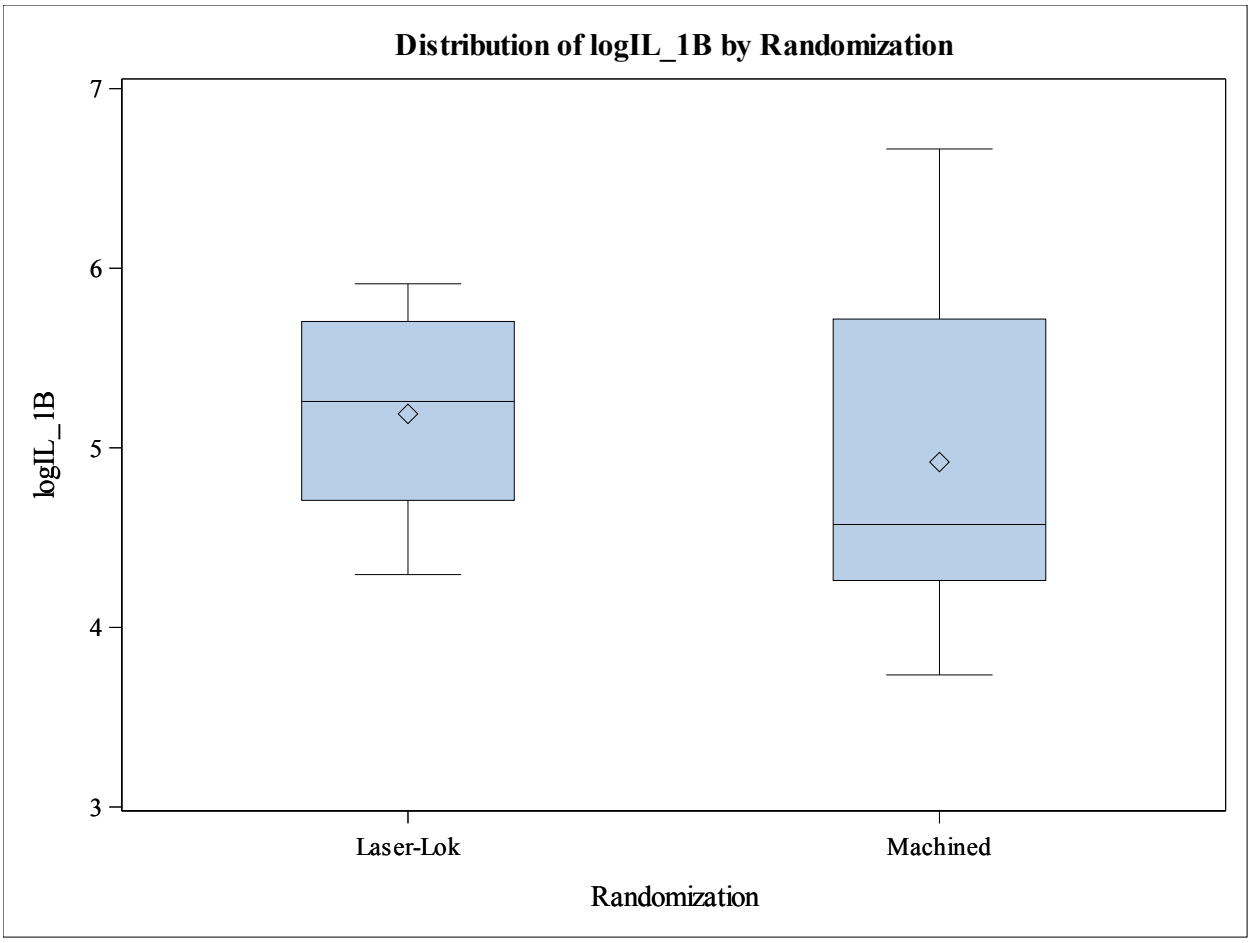
logMMP8b
Laser-Lok
Machined
Randomization


Distribution of logIL_1B by Randomization
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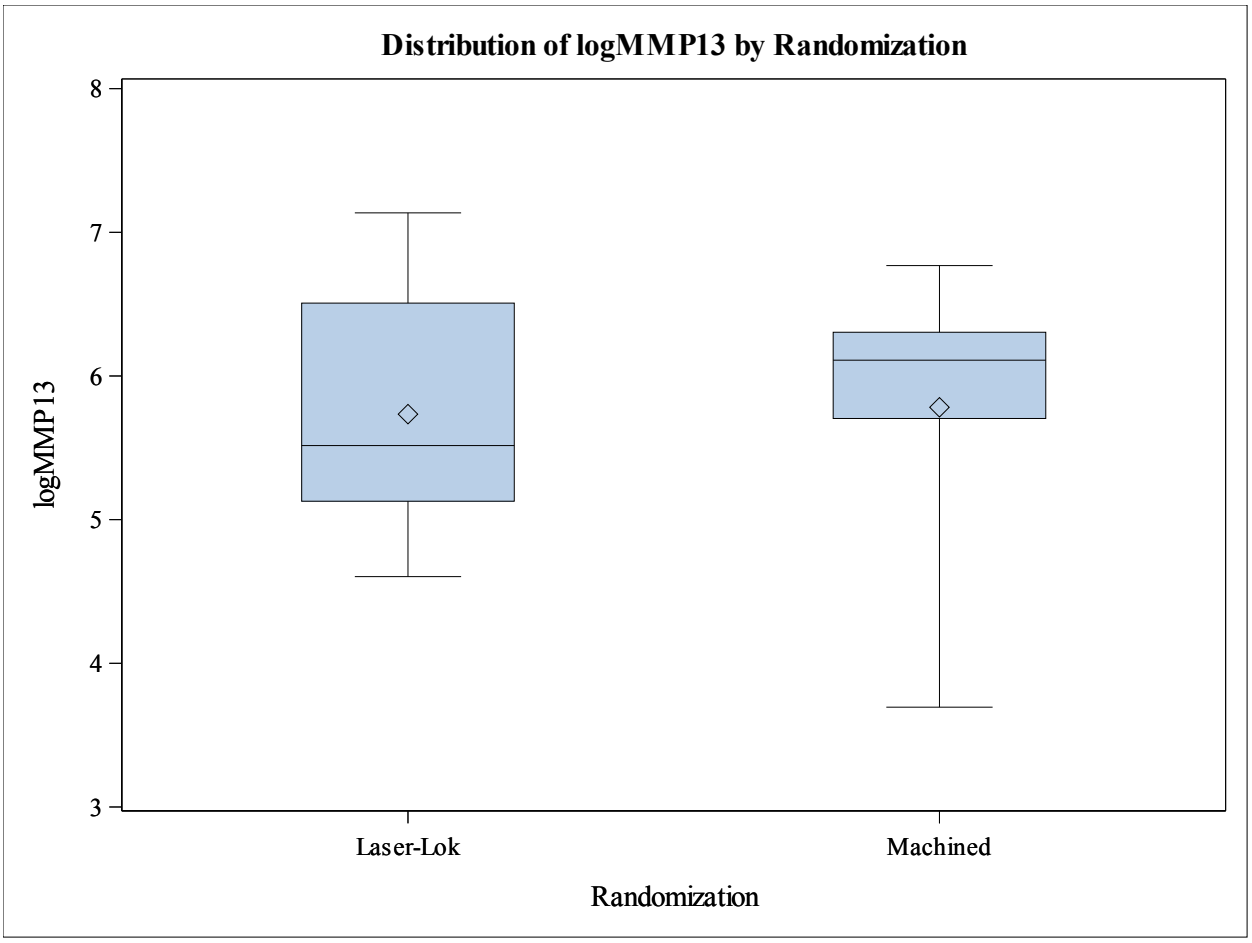
logIL_1B
Laser-Lok
Machined
Randomization


Distribution of logMMP13 by Randomization
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4
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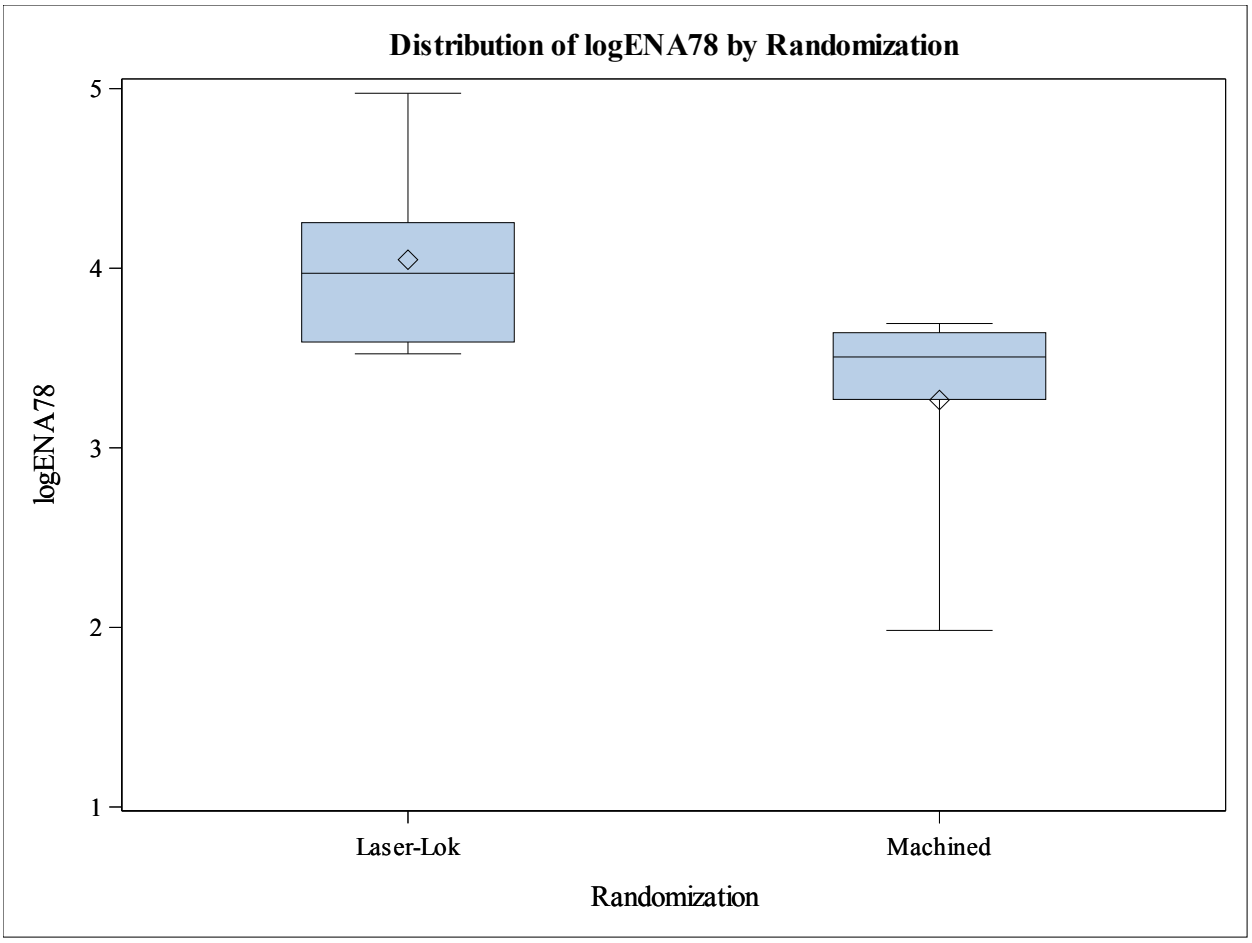
logMMP13
Laser-Lok
Machined
Randomization


Distribution of logENA78 by Randomization
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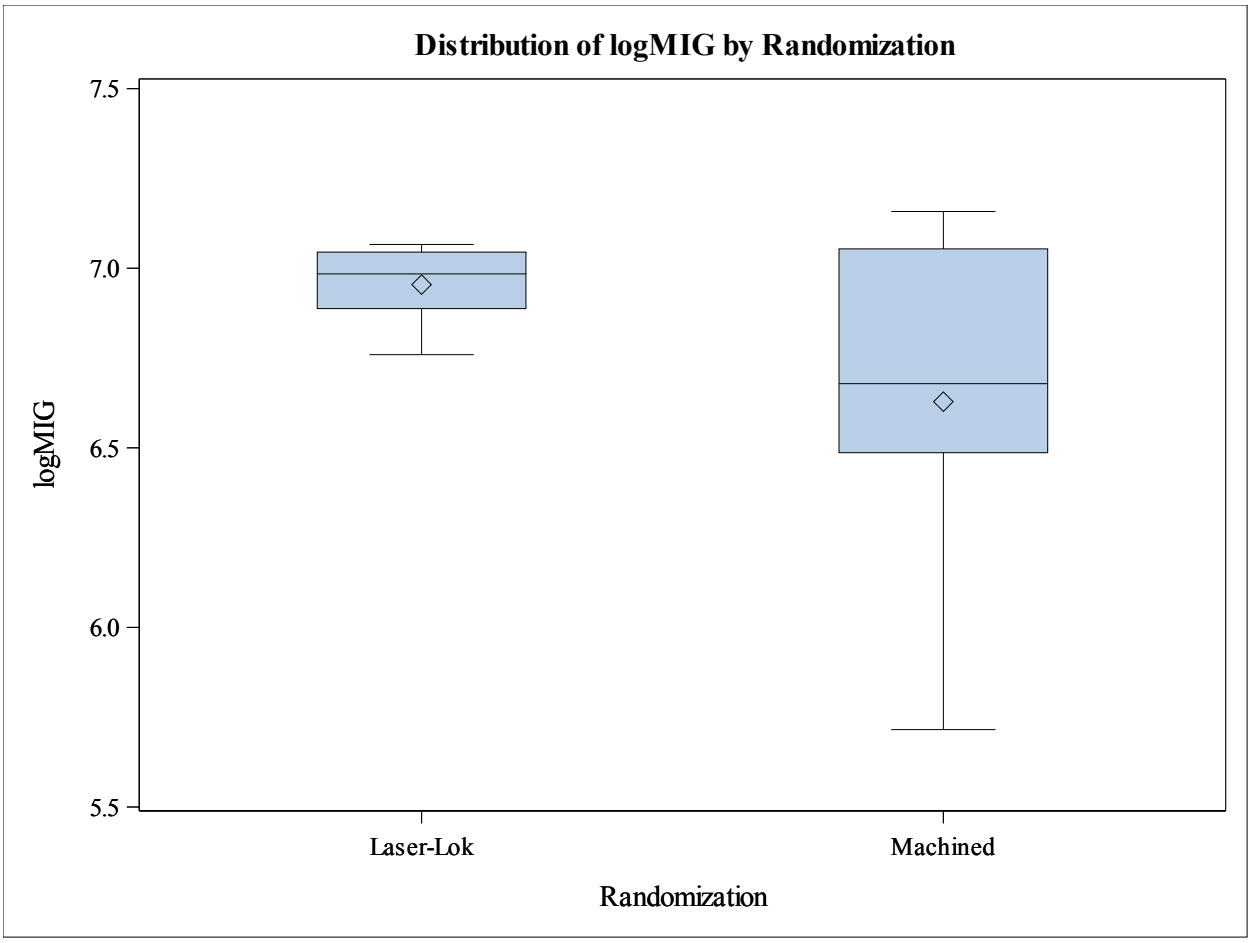
3
2
1
logENA78
Laser-Lok
Machined
Randomization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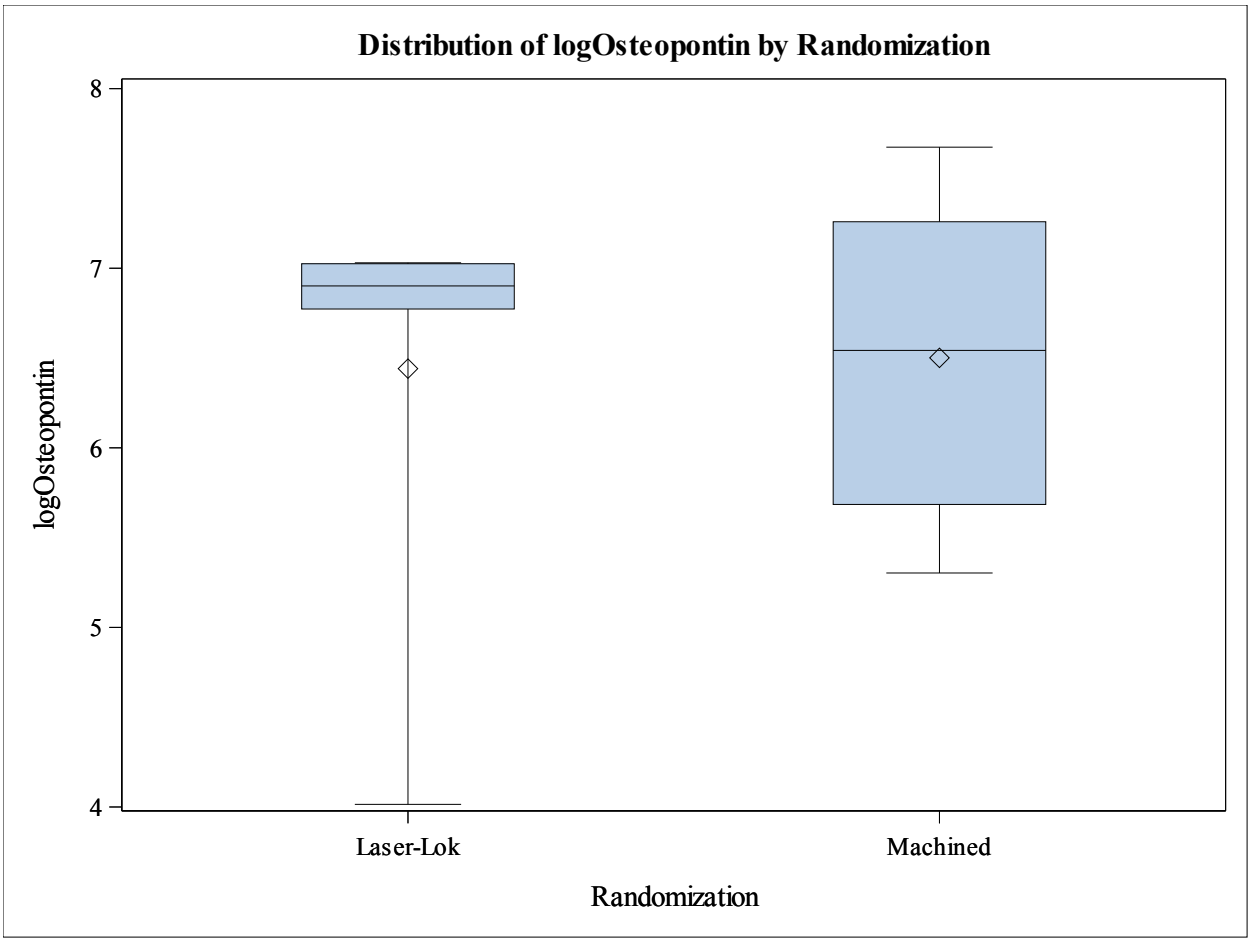
logMIG
Laser-Lok
Machined
Randomization


Distribution of logOsteopontin by Randomization
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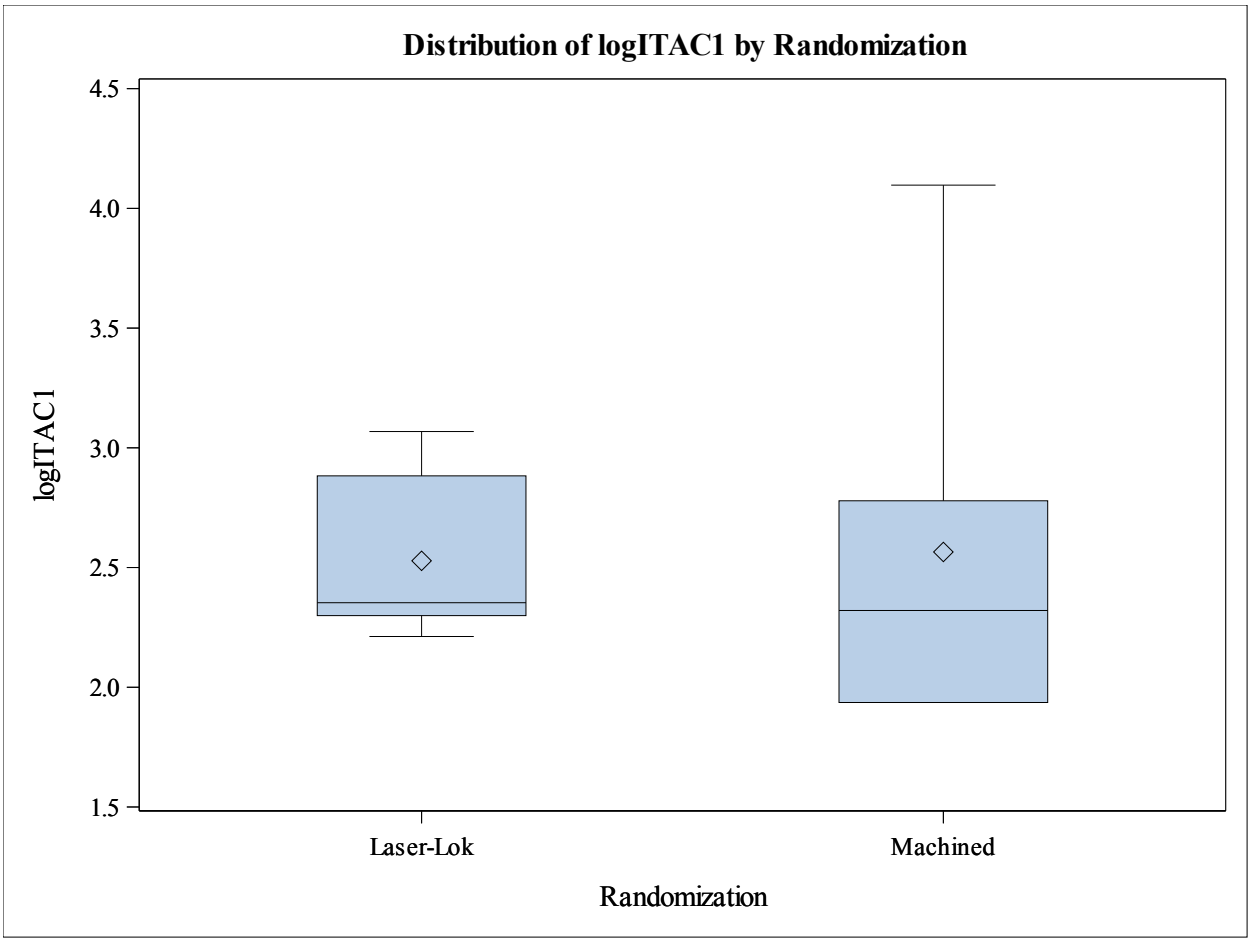
6
5
4
logOsteopontin
Laser-Lok
Machined
Randomization


Distribution of logITAC1 by Randomization
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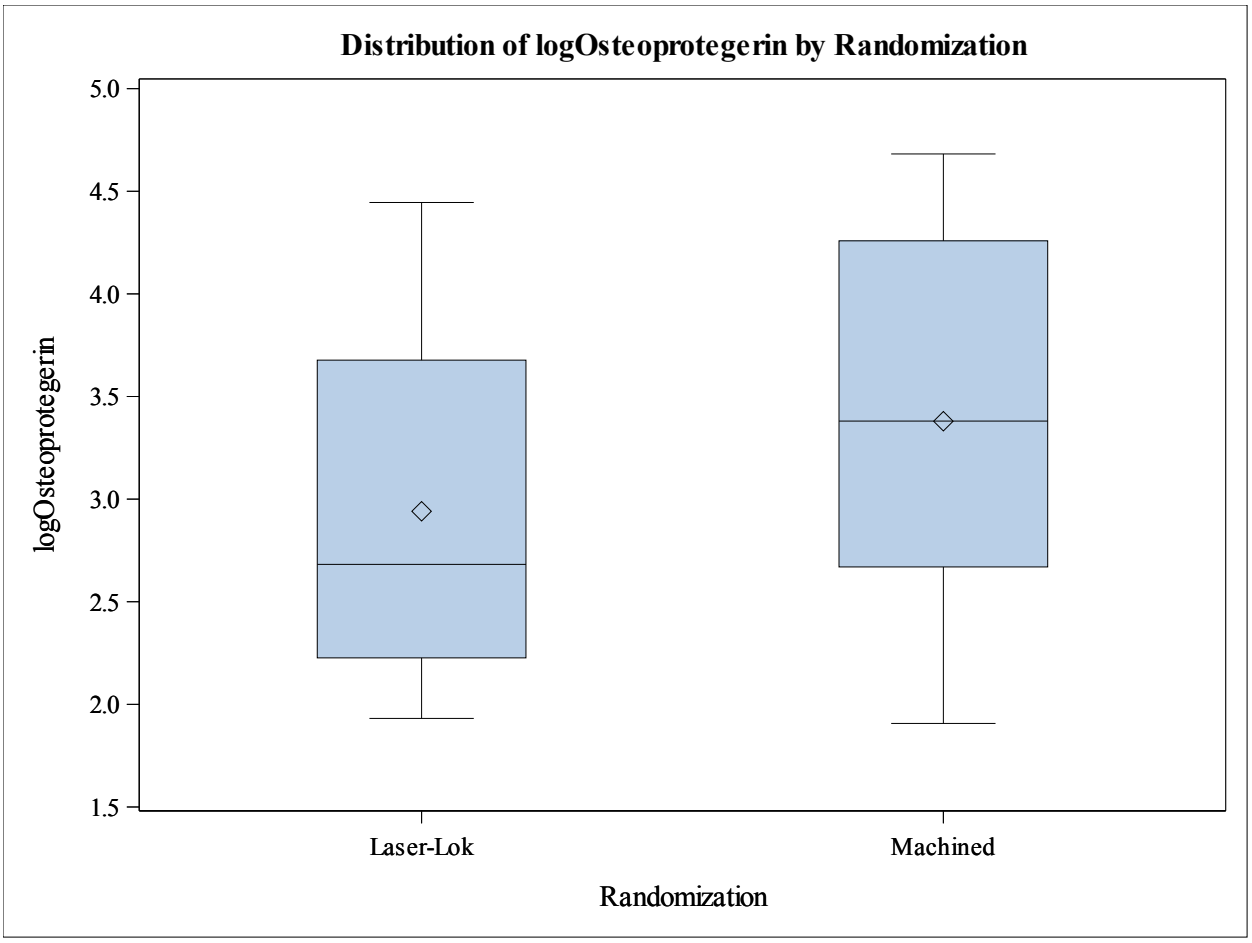
logITAC1
Laser-Lok
Machined
Randomization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3.0
2.5
2.0
1.5
logOsteoprotegerin
Laser-Lok
Machined
Randomization

Group Differences

Table 2. Two-Sample Tests Comparing PICF Cytokine Levels					
		Two Sample T-test		Wilcoxon Rank Sum test	
Measurement	N	P-value	Adjusted P-value	P-value	Adjusted p-value
TNFa	12	0.8881	0.9509	0.9376	0.9376
IL6	12	0.3605	0.7811	0.4862	0.8470
IL8	12	0.0656	0.4264	0.1208	0.7852
MMP1	12	0.1645	0.4277	0.2550	0.8288
IP10	12	0.9509	0.9509	0.9376	0.9376
MMP8b	12	0.1425	0.4277	0.2550	0.8288
IL-1B	12	0.6089	0.9509	0.5864	0.8470
MMP13	12	0.9367	0.9509	0.8146	0.9376
ENA78	12	0.0456	0.4264	0.0927	0.7852
MIG	12	0.1606	0.4277	0.3203	0.8328
Osteopontin	12	0.9256	0.9509	0.6966	0.9056
ITAC1	12	0.9232	0.9509	0.5851	0.8470
Osteoprotegerin	12	0.4673	0.8678	0.4862	0.8470

Conclusion for Laser-Lok and Machined PICF Comparisons

There was not enough evidence of a statistically significant difference between Laser-Lok and machined implants for any of the 13 outcomes measured.

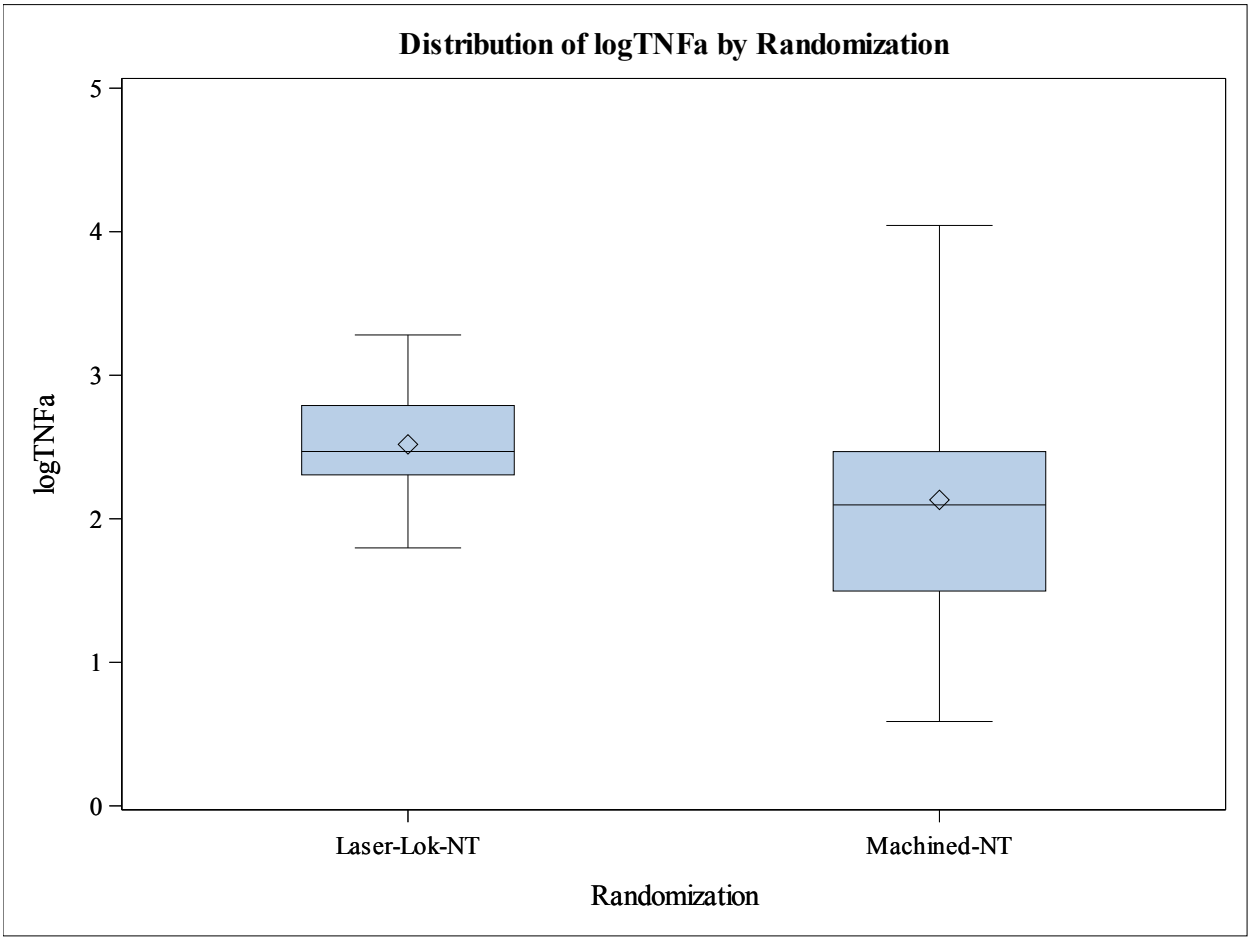
Aim 2: Do the cytokine levels of the Laser-Lok GCF differ from the Machined GCF?

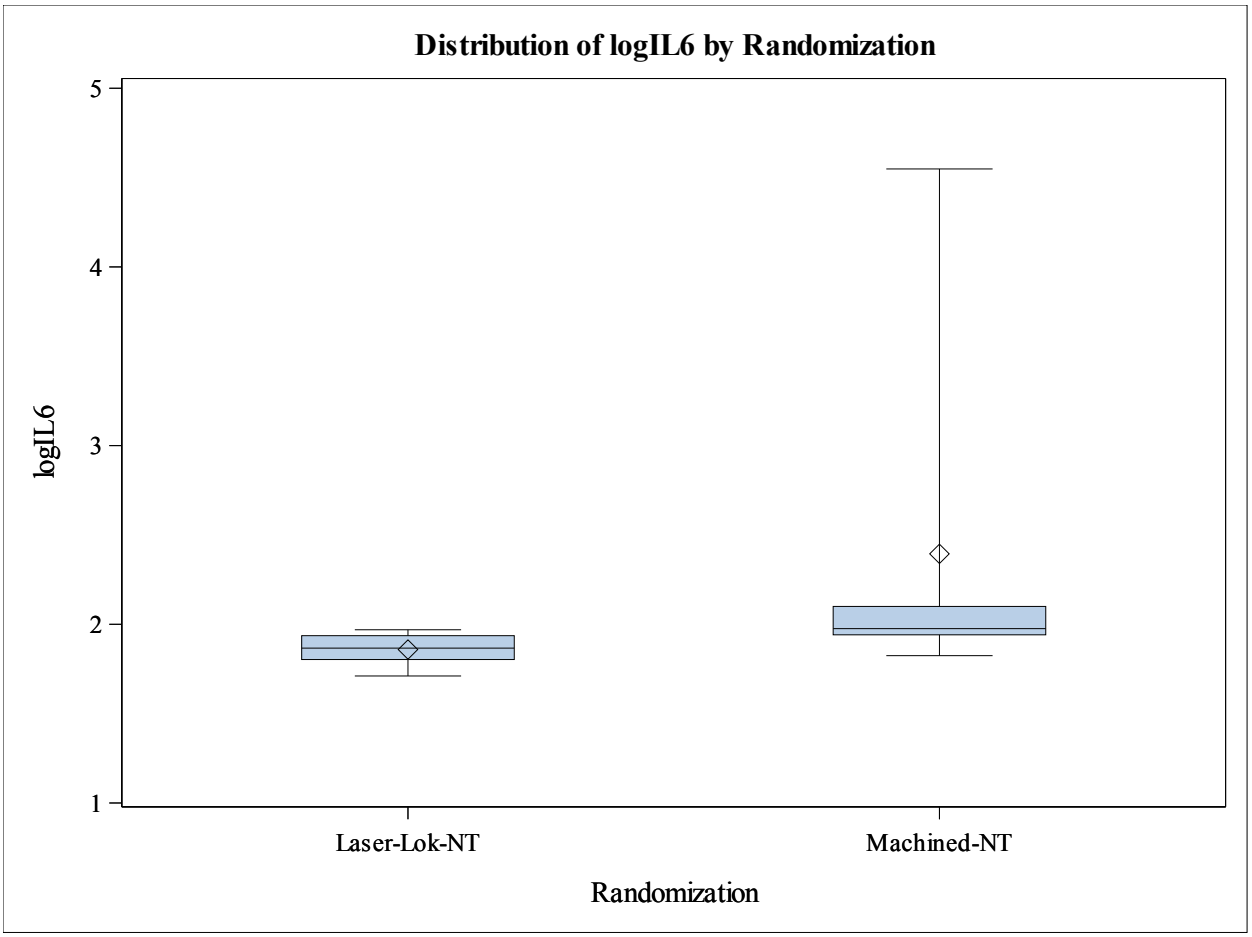
Descriptive Statistics

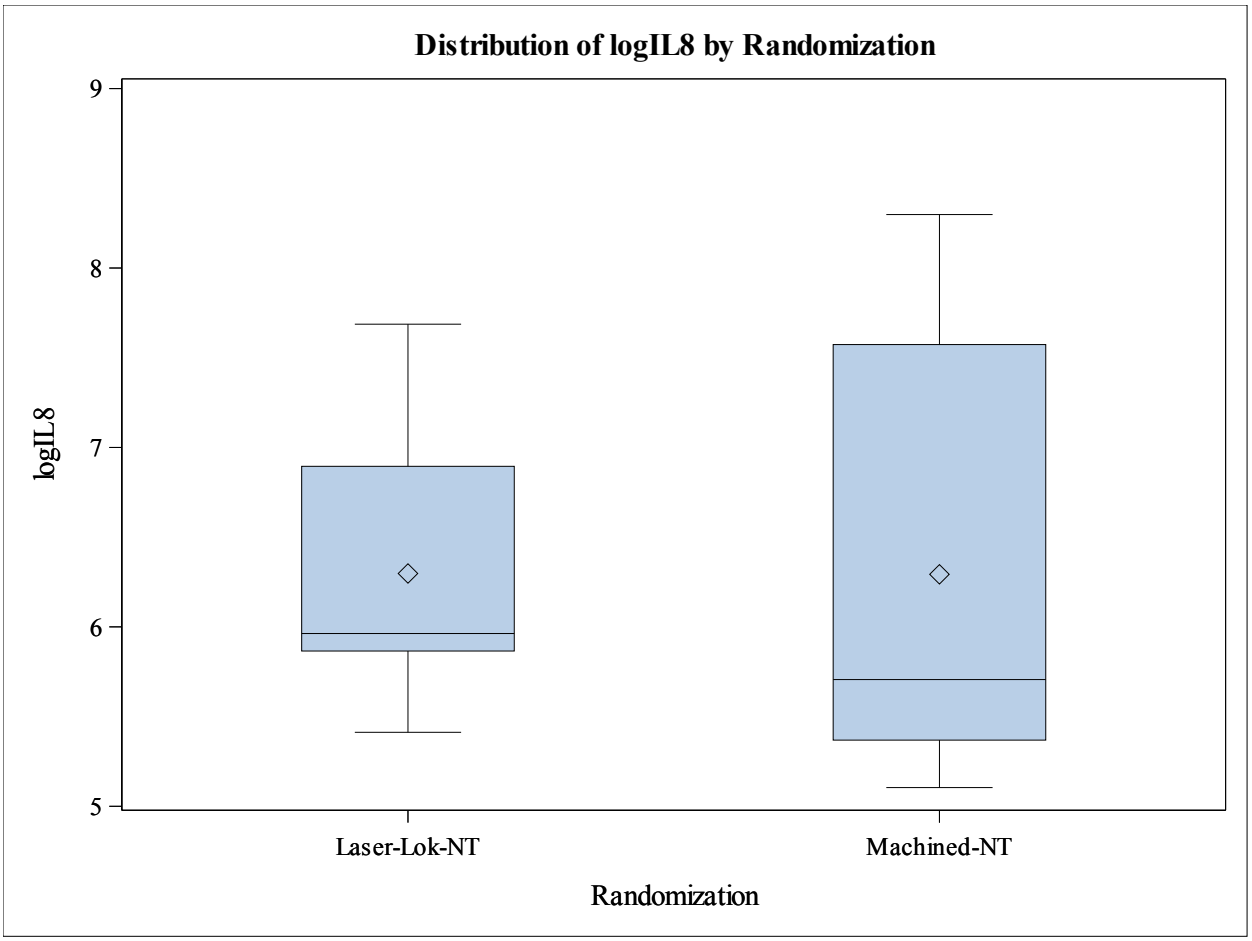
Table 3. Descriptive Statistics for GCF Cytokine Levels

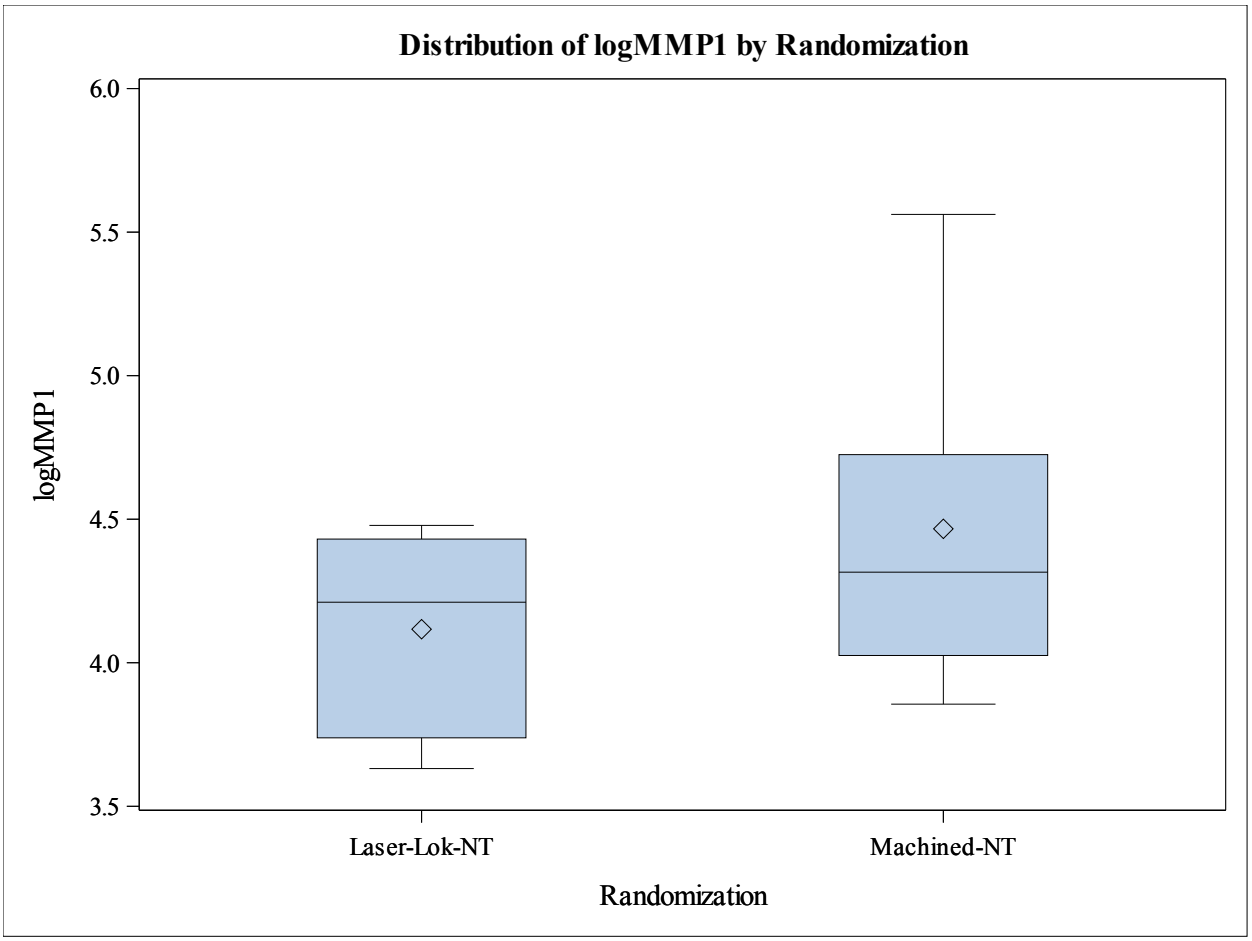
Natural Tooth	Variable	N	Mean	Std Dev	Median	Minimum	Maximum
Laser-Lok-NT	logTNFa	6	2.52	0.50	2.47	1.80	3.28
	logIL6	6	1.86	0.09	1.87	1.71	1.97
	logIL8	6	6.30	0.84	5.96	5.41	7.69
	logMMP1	6	4.12	0.35	4.21	3.63	4.48
	logIP10	6	1.13	0.84	0.94	0.36	2.58
	logMMP8b	6	11.10	0.50	11.03	10.32	11.79
	logIL_1B	6	4.70	0.82	4.91	3.24	5.51
	logMMP13	6	5.63	0.36	5.53	5.15	6.08
	logENA78	6	3.94	0.79	3.61	3.52	5.53
	logMIG	6	6.88	0.18	6.93	6.55	7.03
	logOsteopontin	6	6.82	0.49	6.88	6.28	7.60
	logITAC1	6	2.48	0.20	2.50	2.21	2.69
	logOsteoprotegerin	6	2.84	1.56	2.84	0.51	4.86
Machined-NT	logTNFa	6	2.13	1.15	2.10	0.59	4.04
	logIL6	6	2.39	1.06	1.98	1.82	4.55
	logIL8	6	6.29	1.31	5.71	5.10	8.30
	logMMP1	6	4.47	0.62	4.32	3.86	5.56
	logIP10	6	2.05	2.14	1.64	0.03	5.97
	logMMP8b	6	11.17	0.76	11.43	10.03	11.84
	logIL_1B	6	5.06	0.80	5.05	4.00	6.02
	logMMP13	6	5.18	1.87	5.91	1.39	6.32
	logENA78	6	3.75	0.68	3.62	2.98	4.75
	logMIG	6	6.95	0.32	7.06	6.49	7.31
	logOsteopontin	6	6.56	1.21	6.81	4.23	7.78
	logITAC1	6	2.18	1.01	2.00	1.10	3.97
	logOsteoprotegerin	6	3.69	0.82	3.94	2.42	4.42

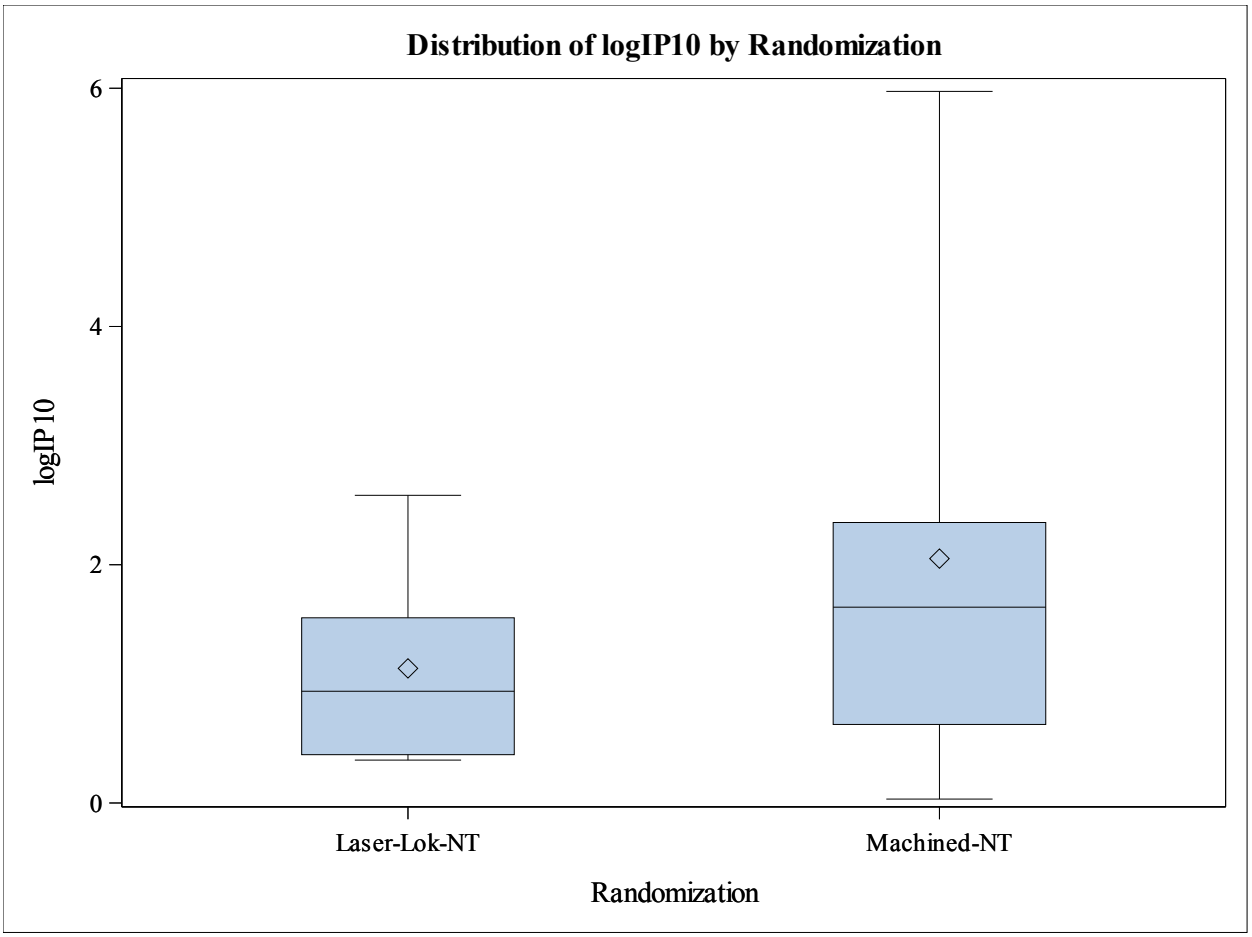
Box Plots

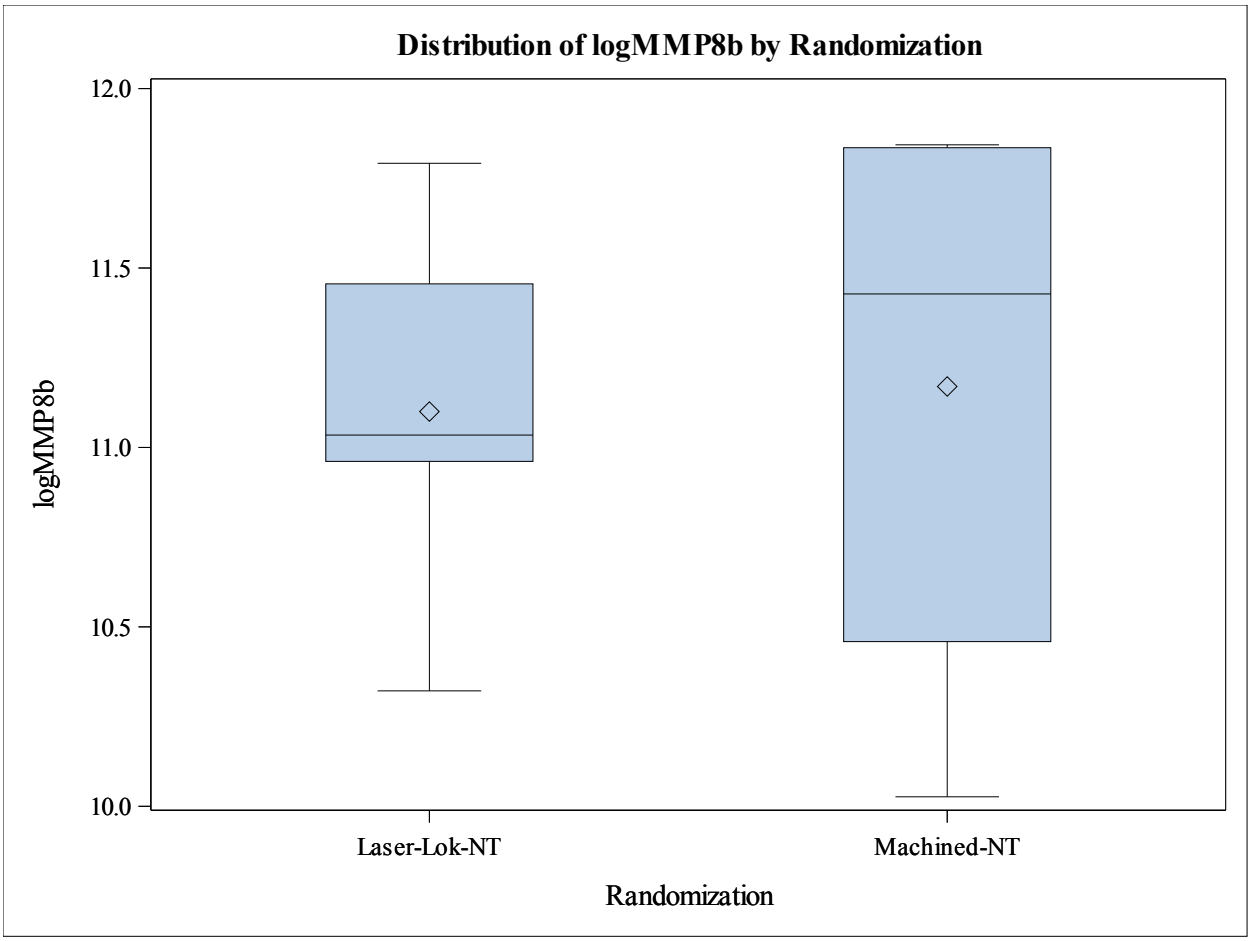

Distribution of logTNFa by Randomization
logTNFa
5
4
3
2
1
0
Laser-Lok-NT
Machined-NT
Randomization

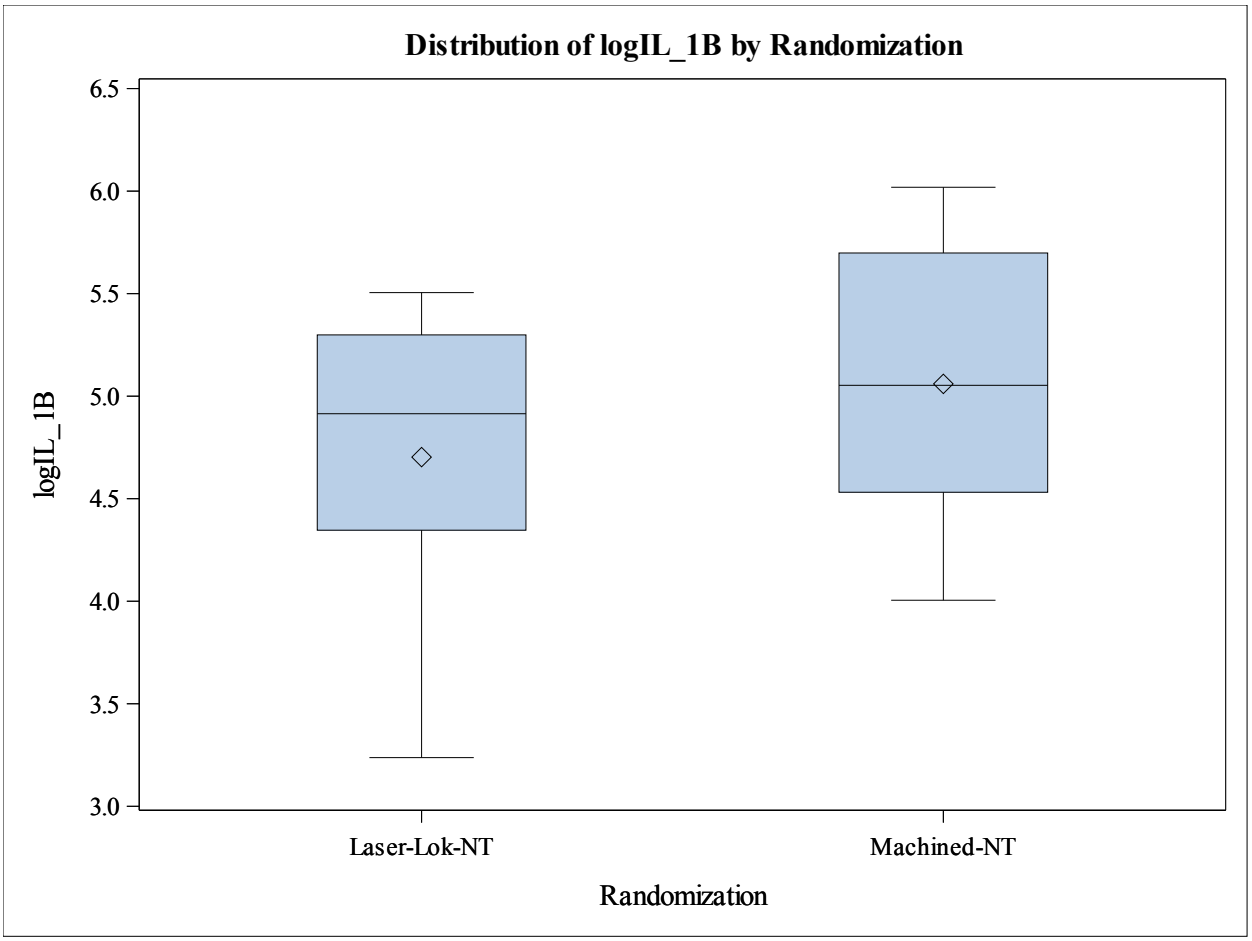

Distribution of logIL6 by Randomization
5
4
3
2
1
logIL6
Laser-Lok-NT
Machined-NT
Randomization

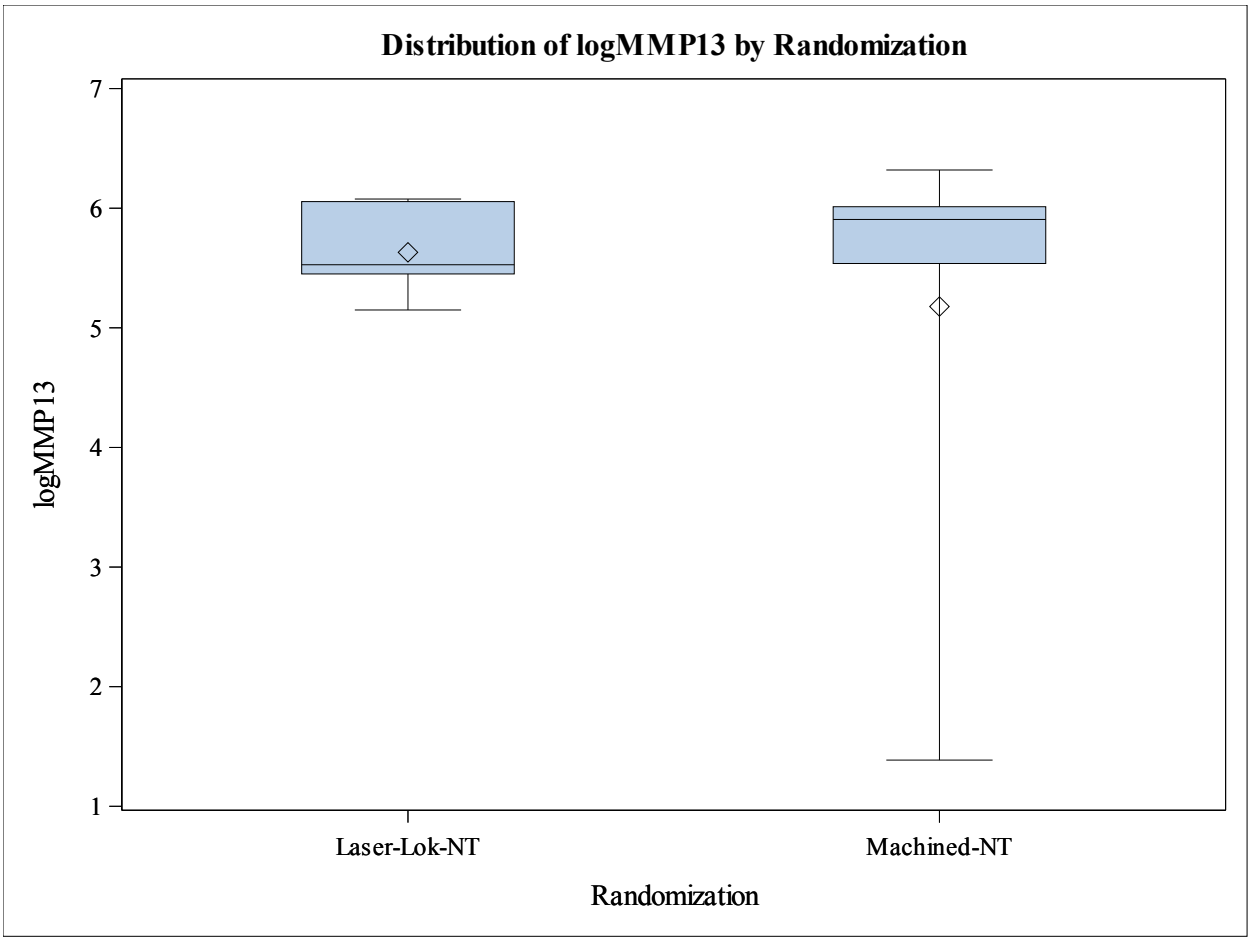

Distribution of logIL8 by Randomization
9
8
7
6
5
logIL8
Laser-Lok-NT
Machined-NT
Randomization

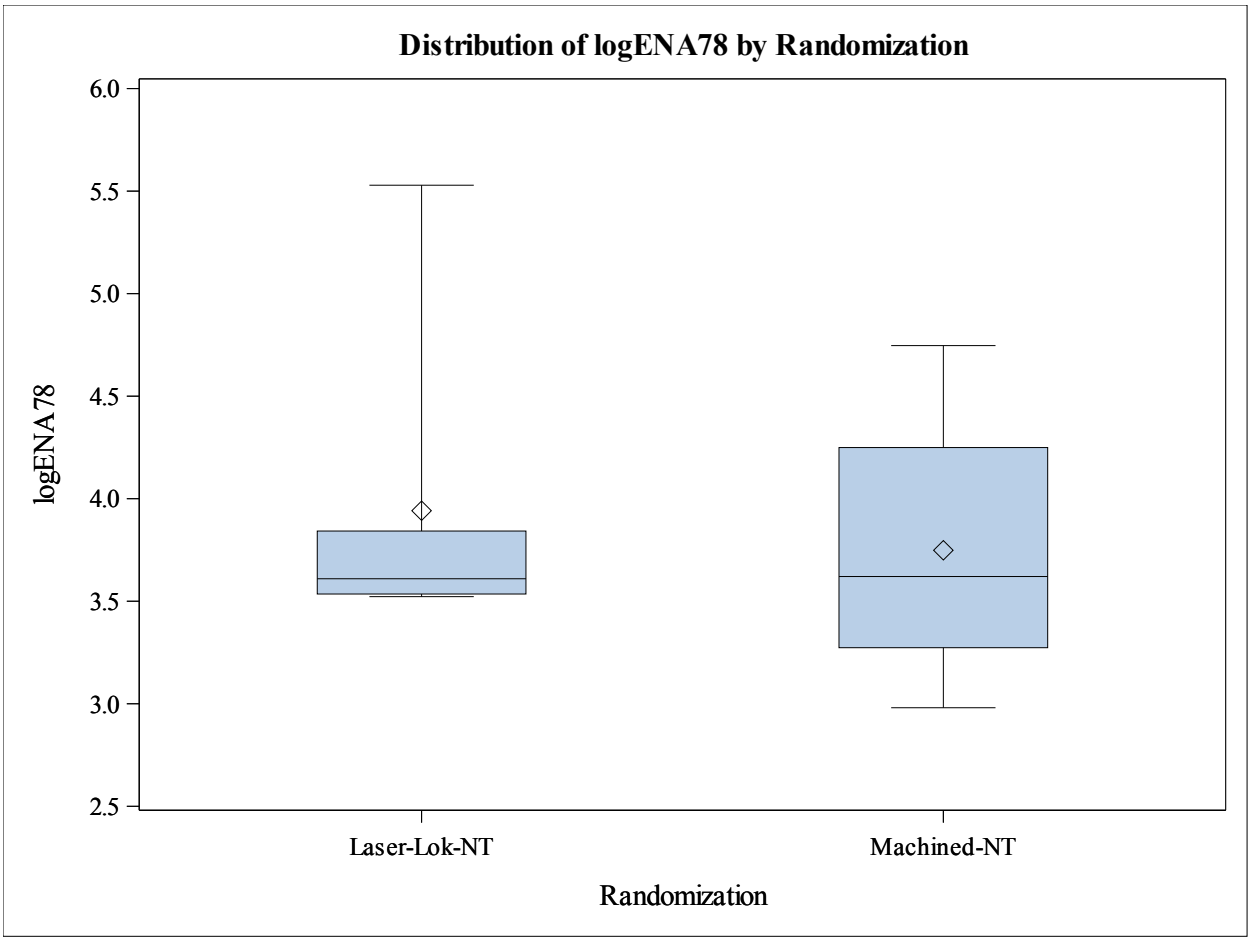

Distribution of logMMP1 by Randomization
6.0
5.5
5.0
4.5
4.0
3.5
logMMP1
Laser-Lok-NT
Machined-NT
Randomization

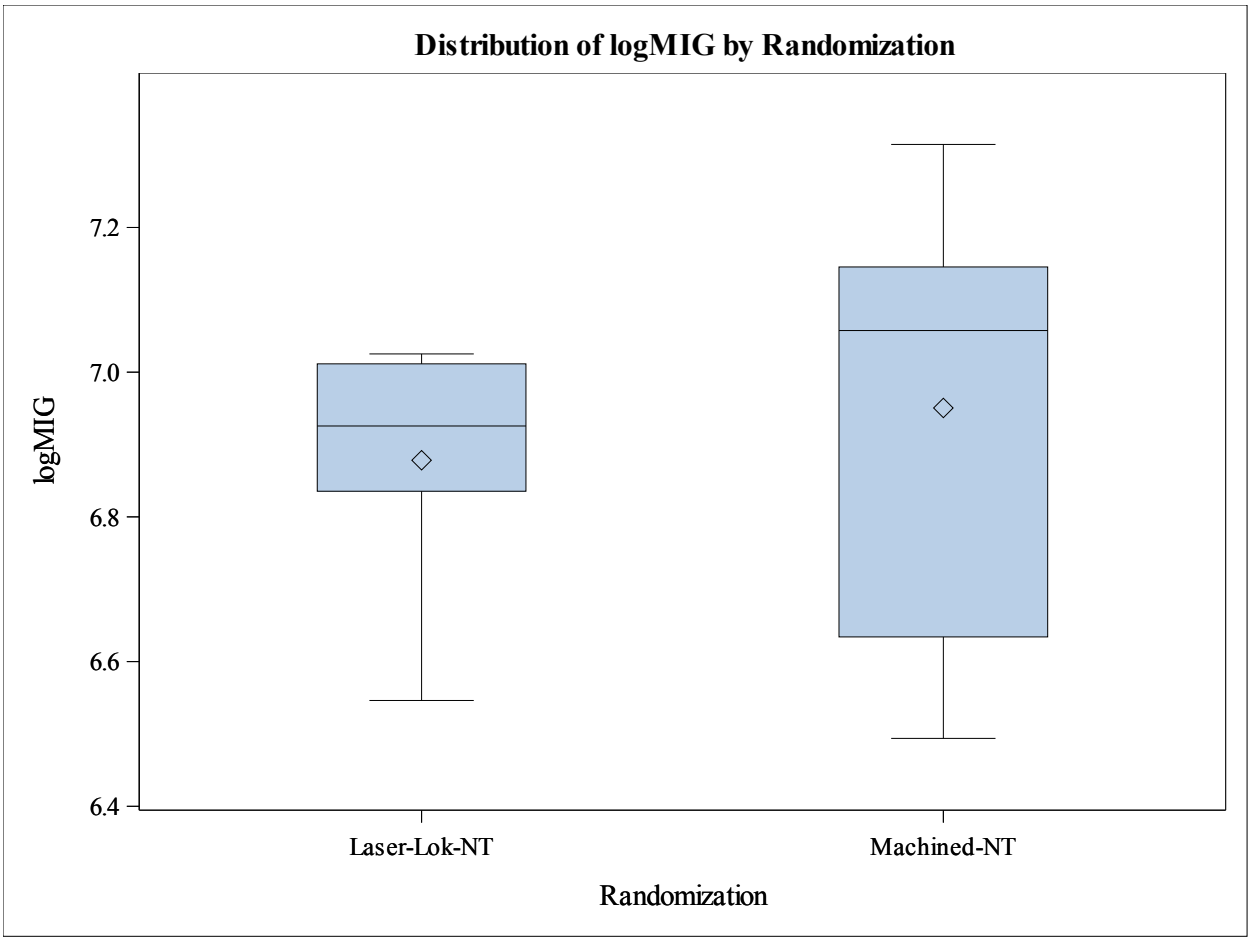

Distribution of logIP10 by Randomization
6
4
2
0
logIP10
Laser-Lok-NT
Machined-NT
Randomization

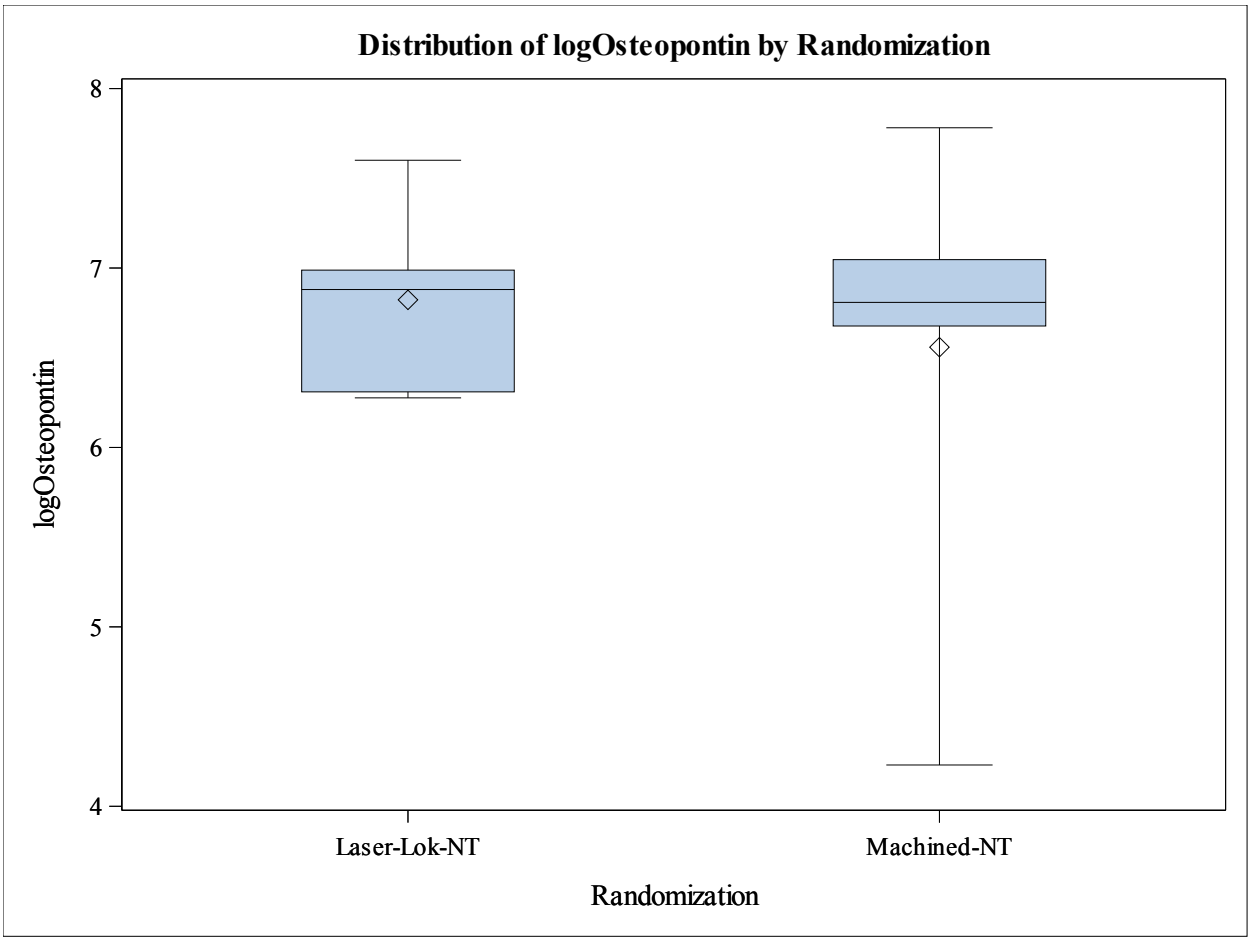

Distribution of logMMP8b by Randomization
12.0
11.5
11.0
10.5
10.0
logMMP8b
Laser-Lok-NT
Machined-NT
Randomization

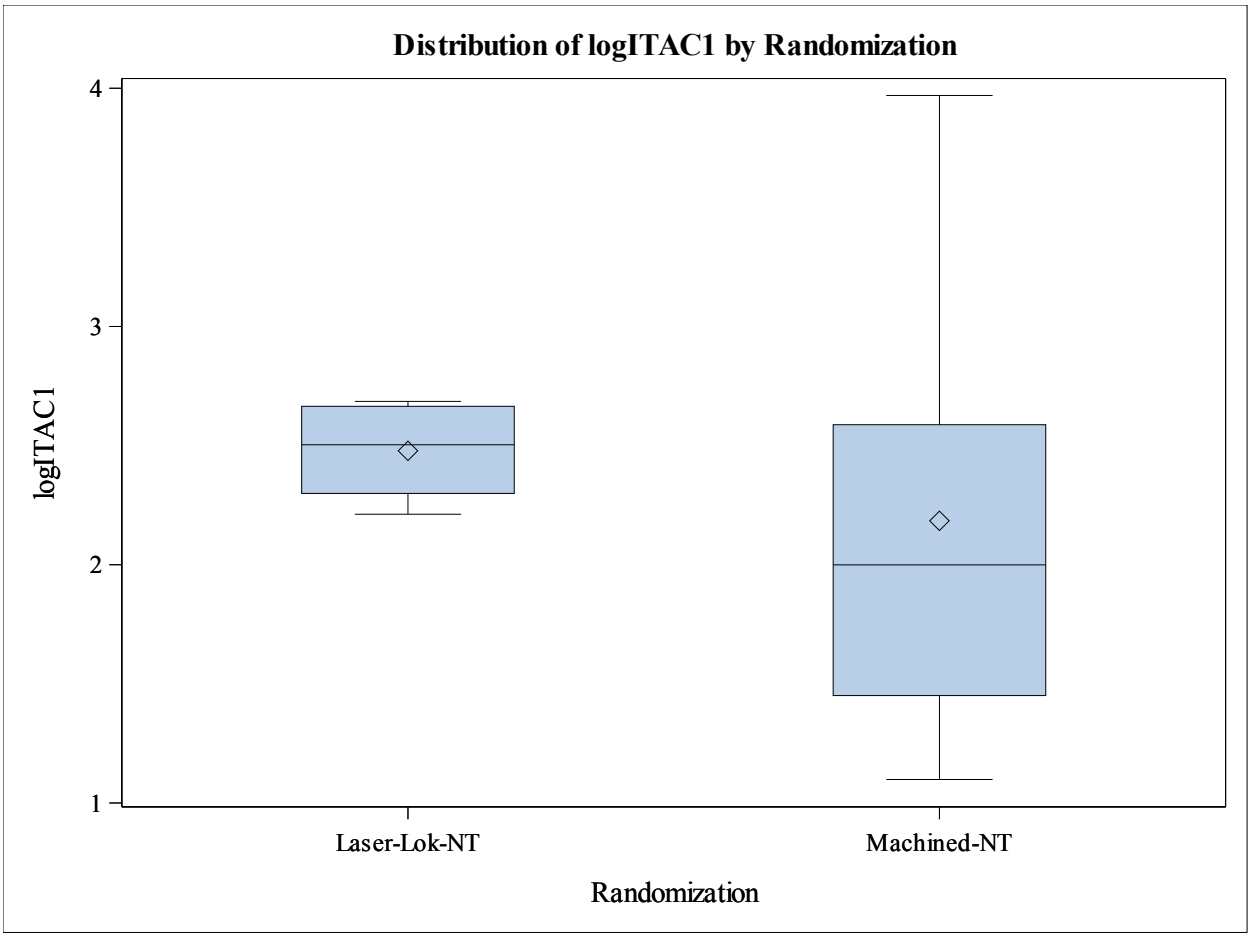

Distribution of logIL_1B by Randomization
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
logIL_1B
Laser-Lok-NT
Machined-NT
Randomization

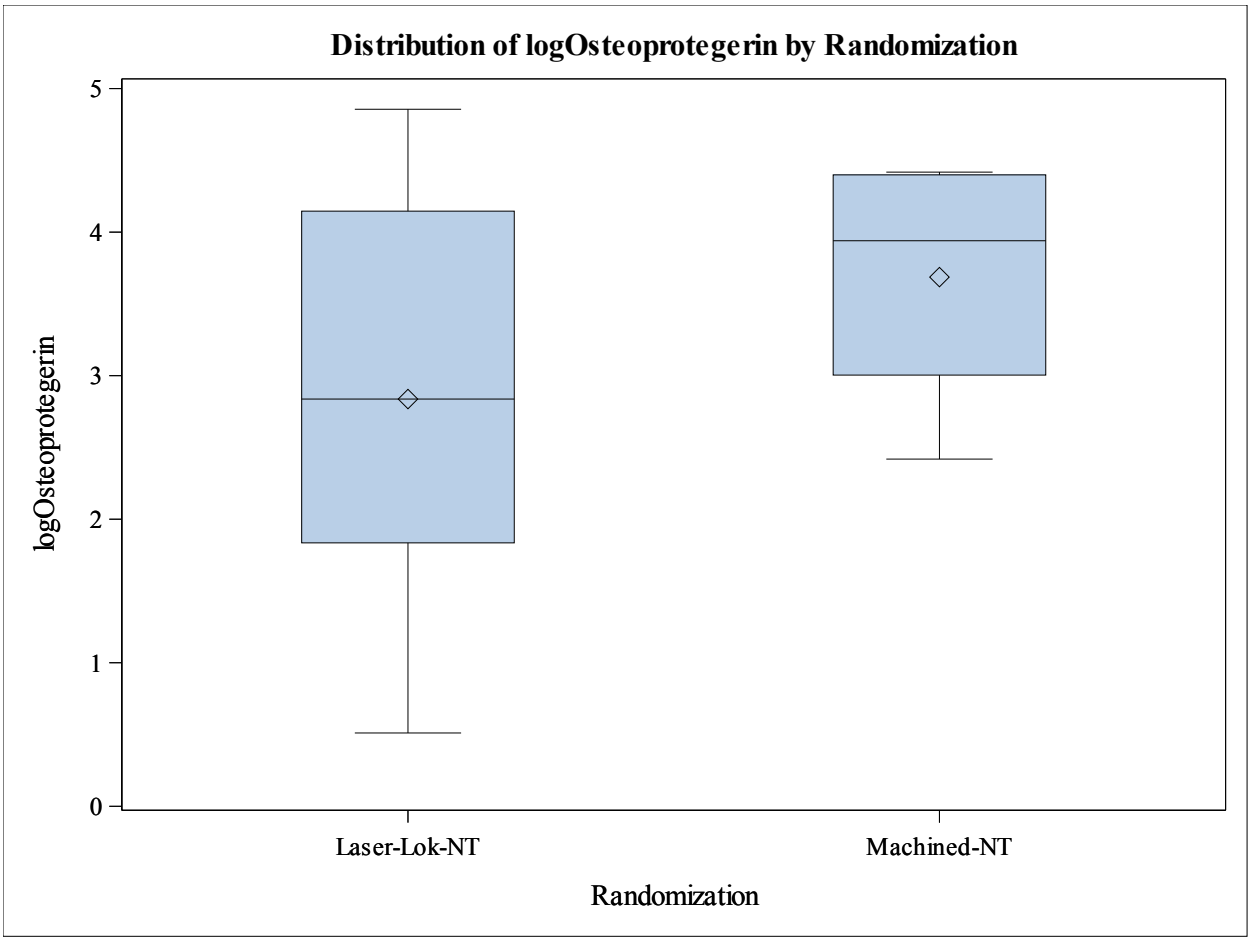

Distribution of logMMP13 by Randomization
7
6
5
4
3
2
1
logMMP13
Laser-Lok-NT
Machined-NT
Randomization


Distribution of logENA78 by Randomization
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
logENA78
Laser-Lok-NT
Machined-NT
Randomization


Distribution of logMIG by Randomization
7.2
7.0
6.8
6.6
6.4
logMIG
Laser-Lok-NT
Machined-NT
Randomization


Distribution of logOsteopontin by Randomization
8
7
6
5
4
logOsteopontin
Laser-Lok-NT
Machined-NT
Randomization


Distribution of logITAC1 by Randomization
4
3
2
1
logITAC1
Laser-Lok-NT
Machined-NT
Randomization


Distribution of logOsteoprotegerin by Randomization
logOsteoprotegerin
5
4
3
2
1
0
Laser-Lok-NT
Machined-NT
Randomization

Group Differences

Table 4. Two-Sample Tests Comparing GCF Cytokine Levels					
		Two Sample T-test		Wilcoxon Rank Sum test	
Measurement	N	P-value	Adjusted P-value	P-value	Adjusted p-value
TNFa	12	0.4672	0.7783	0.3203	0.7547
IL6	12	0.2455	0.7783	0.0927	0.7547
IL8	12	0.9943	0.9943	0.4862	0.7547
MMP1	12	0.2584	0.7783	0.4862	0.7547
IP10	12	0.3485	0.7783	0.6966	0.7547
MMP8b	12	0.8545	0.9257	0.5864	0.7547
IL-1B	12	0.4635	0.7783	0.4862	0.7547
MMP13	12	0.5739	0.7783	0.5864	0.7547
ENA78	12	0.6586	0.7783	0.6966	0.7547
MIG	12	0.6387	0.7783	0.4862	0.7547
Osteopontin	12	0.6308	0.7783	0.9376	0.9376
ITAC1	12	0.5022	0.7783	0.2007	0.7547
Osteoprotegerin	12	0.2660	0.7783	0.3195	0.7547

Conclusion for Laser-Lok and Machined GCF Comparisons

There was not enough evidence of a statistically significant difference between Laser-Lok natural teeth and machined natural teeth for any of the 13 outcomes measured.

Cluster Analysis

Implant Values

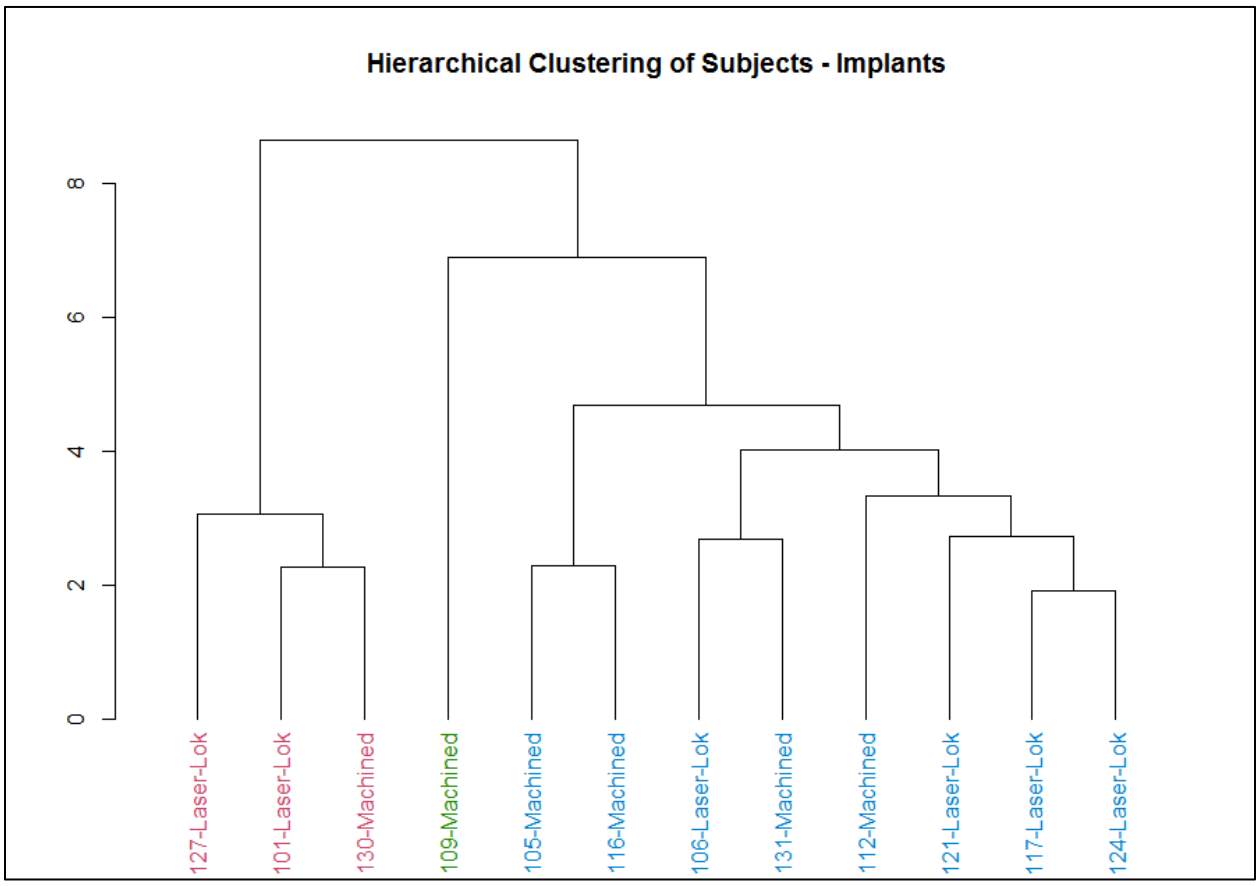


Figure 1. Implants: Clustering of subjects based on Euclidean Distance and Log Scaled Values

Clustering of subjects and the resulting dendrogram is given in Figure 1. The tree was partitioned into three clusters and labeled using patient IDs and group assignments.

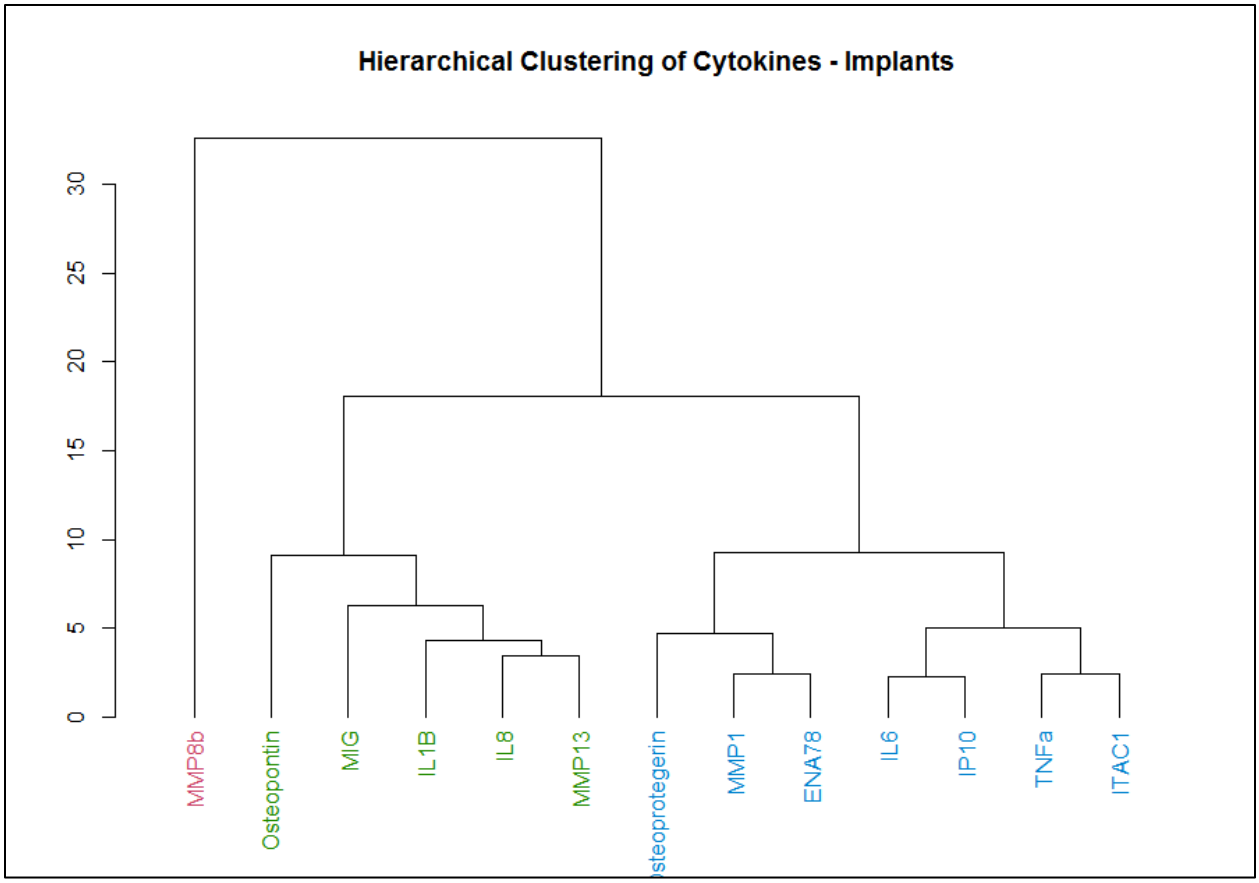


Figure 2. Implants: Clustering of Cytokines based on Euclidean Distance and Log Scaled Values

Cytokines were clustered and the resulting dendrogram is given in Figure 2. The tree was partitioned into three clusters and labeled using the cytokine names.

Natural Teeth Values

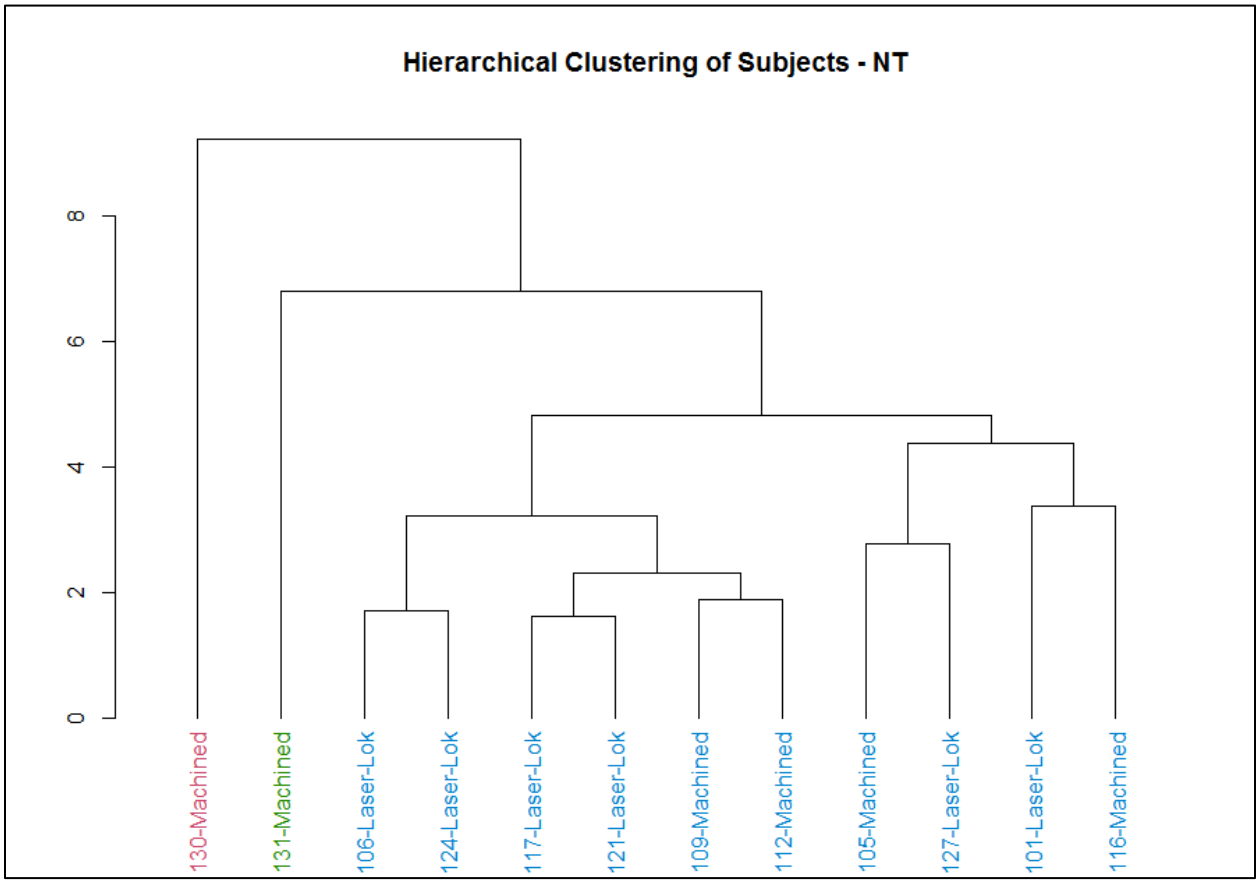


Figure 3. Natural Teeth: Clustering of subjects based on Euclidean Distance and Log Scaled Values

Clustering of subjects and the resulting dendrogram is given in Figure 1. The tree was partitioned into three clusters and labeled using patient IDs and group assignments.

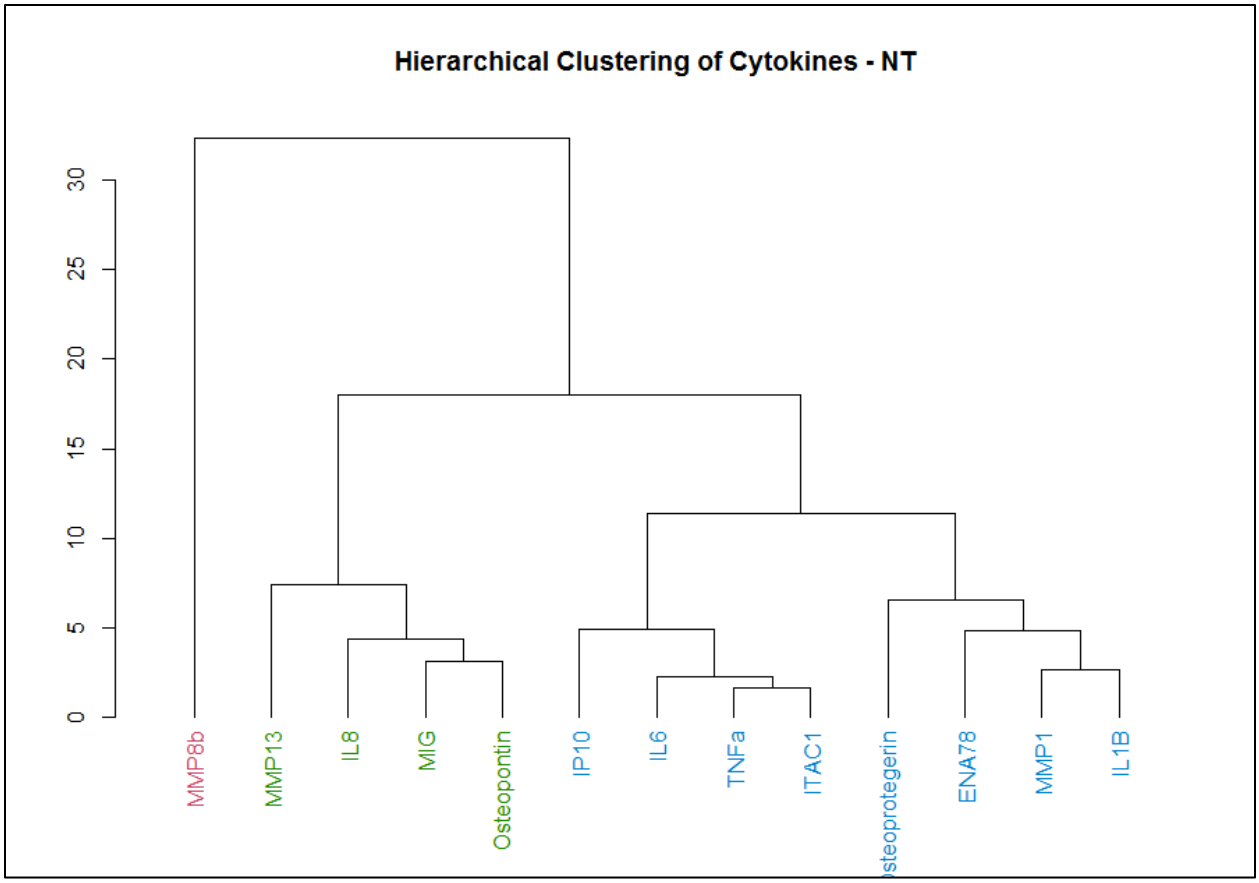


Figure 4. Natural Teeth: Clustering of Cytokines based on Euclidean Distance and Log Scaled Values

Cytokines were clustered and the resulting dendrogram is given in Figure 2. The tree was partitioned into three clusters and labeled using the cytokine names.