Osteopathic manipulative therapy and its role to enhance the immune response of the body, modification the plasma level of cytokines, Cortisol and Serotonin ; and its positive effect on the anti – infection, anti -stress and anti- inflammatory responses.

 Osteopathic medicine is based on the premise that the primary role of it is to facilitate the body's inherent ability to heal itself. These manipulative therapies have been successfully used by osteopaths for more than a hundred years in order to treat dysfunctions of the neuromusculoskeletal, lymphatic, or vascular tissue. As described in Foundations for osteopathic medicine, some of the techniques relevant to osteopathy include:1) soft-tissue techniques that increase muscle relaxation and circulation of body fluids; and 2) isometric and isotonic techniques that focus on restoring physiological movements and altered joint mechanisms. The goal of osteopathic manipulative medicine is to evaluate biomechanical (somatic) dysfunction and, through the application of osteopathic manipulative treatment (OMT) to promote healing in patients with these disorders. Objectively evaluating somatic dysfunction and how it changes after OMT has been challenging to researchers and clinicians alike.
 Manipulative therapies aimed to increase lymphatic flow, such as thoracic or abdominal lymphatic pump, have been extensively used in osteopathic medicine. In particular, these techniques are proposed to treat patients with asthma, edema and certain pulmonary infections since an increase in the lymphatic flow may enhance filtering and removal of fluid, inflammatory mediators, and waste products from interstitial spaces.

 It has been claimed that osteopathic manipulative treatment (OMT) is able to enhance the immune response of individuals. In particular, it has been reported that OMT has the capability to increase antibody titers, enhance the efficacy of vaccination, and upregulate the numbers of circulating leukocyte. Recently, it has been shown in human patients suffering chronic low back pain, that OMT is able to modify the levels of cytokines such as IL-6 and TNF-a in blood upon repeated treatment. Further, experimental animal models show that lymphatic pump techniques can induce a transient increase of cytokines in the lymphatic circulation. In more recent years, exciting studies on the effect of OMT on immune parameters have been performed. Firstly, three publications from the Hodge lab have shown that abdominal lymphatic pump can significantly modify the leukocyte population in lymphatic circulation. By using an animal experimental dog model, these researchers showed that OMT can exert a mechanotransduction stimulus that is capable of modifying immune parameters. In addition, a recent publication from the same lab showed that thoracic and abdominal lymphatic pump techniques were able to reduce Streptococcus pneumonia colony forming units in the lungs of rats with acute pneumonia .Although no identification of the particular mechanism responsible for this effect has yet been determined, this data clearly highlights the capability of OMT to enhance protection against infection. Further, two recent research studies by Licciar done et al on human patients suffering chronic low back pain showed that repeated OMT treatment was able to modify the levels of TNFa in circulation. Also, randomized, controlled clinical trial comparing a standardized lymphatic pump protocol with a light touch protocol determined that OMT can induce a rapid and significant decrease in the platelet levels in elder patients .Finally ,many reports evidence in

humans the positive effect of manipulation, in particular of osteopathic lymphatic techniques in normal as well in pathological conditions. In animals too, lymphatic treatment has been reported to induce changes of lymphatic flux and to mobilize inflammatory mediators thus determining positive effects on the immune system.

In one research that became published by International jurnal of Osteopathic Medicine on March 2013, they noticed that Osteopathic lymphatic techniques reduces cortisol plasma level in rats after repeated treatments. Based on this research blood cell count did not show differences at baseline and at the end of the experiment both in control and in treated rats. Blood serum chemistry was also unchanged. In particular, blood urea nitrogen (BUN) and serum creatinine (expression of renal function), alanine transaminase (ALT) and aspartate transaminase (AST) (indicative of hepatic cell integrity) were normal and did not change after lymphatic treatment. No changes in plasmatic level of cytokines were found after the five days lymphatic treatment. On the contrary and unexpectedly, cortisol level was significantly reduced in rats that were treated with lymphatic techniques. In untreated rat group cortisol level was, however, minimally increased. These findings were confirmed by the results of the replicated study.

This study demonstrates that repeated lymphatic treatment is a safe procedure. Moreover, lymphatic techniques induce reduction in cortisol level, which may be related to an anti-stress effect.

In another research that became conducted by Heritage college of Osteopathic Medicine of Ohio University on March 2014, they decided to investigate in healthy individuals the capacity of OMT to induce a rapid modification of the levels of cytokines and leukocytes in circulation. Human volunteers were subjected to a mixture of lymphatic and thoracic OMT, and shortly after the levels of several cytokines were evaluated by protein array technology and ELISA multiplex analysis, while the profile and activation status of circulating leukocytes was extensively evaluated by multicolor flow cytometry. In addition, the levels of nitric oxide and C-reactive protein (CRP) in plasma were determined.

In this study, their results show that OMT was not able to induce a rapid modification in the levels of plasma nitrites or CRP or in the proportion or activation status of central memory, effector memory or native CD4 and CD8 T cells. A significant decrease in the proportion of a subpopulation of blood dendritic cells was detected in OMT patients. Significant differences were also detected in the levels of immune molecules such as IL-8, MCP-1, MIP-1a and most notably, G-CSF. Thus, OMT is able to induce a rapid change in the immunological profile of particular circulating cytokines and leukocytes.

In a series of complementary studies they investigated the effect of OMT on the circulating levels of cytokines that were modified in their first series of qualitative studies they were able to detect a significant increase in the plasma levels of MIP-1a in the OMT group compared with pre-treatment values, 30 minutes post treatment .a significant increase in plasma levels of IL-8 was observed in the OMT group compared to basal values 60 min after treatment. In addition, the levels of MCP-1 were also significantly higher in the OMT group compared to sham controls at 60 min post treatment, mostly due to a decrease in the levels of this cytokine in normal controls. This again draws attention to the effect of repeated venipuncture on hematological parameters. More importantly, plasma levels of G-CSF, an inducer of monocyte production by the bone marrow, were significantly up regulated only in the OMT group both at 30 and 60 min post treatment .This further highlights the effects of OMT on the monocyte-dendritic population,

since the CD16 DC population is considered to be derived from CD16 monocytes, and shares characteristics with them. In all, some of this cytokine data is in line what we have observed in our first series of experiments and further strengthen our data pointing towards an immune modulatory role of OMT. In order to investigate the effect of OMT on the level of circulating metabolites and leukocytes, healthy volunteers were subjected to a mixture of lymphatic and hepatic pump treatments while a sham group received a ''light touch'' treatment. In order to determine the basal levels of circulating metabolites and leukocytes1 h prior treatment blood was extracted from the volunteers. To evaluate immediate response to OMT, blood was drawn at 5 and 30 min post treatment .They investigated the levels of CRP.

The levels of this acute response protein are dramatically elevated upon an inflammatory stimulus, but since its synthesized de novo by the liver, very important changes in the levels of this molecular usually observed after 4 h of stimulation. Nevertheless, taking into account some reports that indicate a small but significant increase of CRP at very early time points after stimulation, they decided to evaluate if OMT was able to induce a modification of this factor in circulation. No differences were observed between OMT and sham groups in the circulating levels of CRP at 5 and 30 min post-treatment. As described above, some reports indicate that OMT could be capable of modifying the levels of circulating leukocytes, but they were not able to observe significant differences in the number or proportion of different leukocyte populations immediately after OMT. Further, flow cytometry analysis did not reveal differences in the levels of NK cells or CD3T cells present in the PBMCs samples of OMT or sham treated volunteers 30 min post treatment .These data does not contradict previous observations taking into account that they were focusing in very early responses, while other studies have focused on later responses. In order to further investigate the rapid response of the immune system to OMT, they evaluated the levels of various chemokines, cytokines and growth factors in the plasma of OMT and sham treated subjects using antibody array technology (Human Inflammatory Array; Ray Biotech). Plasma samples (pre- treatment and 30 min post-treatment) from 4 OMT and 4 control subjects were compared for changing levels of 40 different factors associated with inflammatory responses. Expression levels of four cytokines (eotaxin, eotaxin-2, IL-10, IL-16) were significantly increased (1.56 ×increase at 30 minutes vs. pre-treatment level) only in OMT subjects (4 of 4 OMT subjects vs. 0 of 4 control subjects) .Further, there was a general pattern of increase for multiple other factors in 3 of 4 OMT subjects (G-CSF, MIP-1α, sTNFR1), or 2 of 4 OMT subjects (GM-CSF, IL-1α, IL-2, IL-6, IL-8, IL-11, MCP-1, TNF-β) vs. 0 of 4 control subjects. Large variations in level of cytokine expression by individual subjects within each study group were observed with some cytokines. Similar rapid responses in the levels of cytokines have been observed in an experimental model, in which dogs subjected to lymphatic pump techniques showed a rapid discharge of cytokines in the lymphatic circulation .It is noteworthy to comment that these qualitative observations on a limited number of samples suggested the possibility of a rapid change in the cytokine milieu in response to OMT.

Taking into account this qualitative modification in the levels of some circulating cytokines without affecting leukocyte populations, they decided to change their time points for blood collection after OMT, selecting 30 min and 60 min post-treatment. They hypothesize that this would encompass the possibility of a modification in leukocyte levels in response to changes in the cytokine profile. A small but significant decrease in the levels of nitric oxide was observed only in the OMT group at 1 h post-treatment when compared with the values obtained at pre-treatment.

Table 1. Frequency of Significant Post-treatment Increases in Cytokine Expression.

Analyte	Sham	OMT	Analyte	Sham	OMT
EOTAXIN	0	4	Timp-2	0	0
EOTAXIN-2	0	4	IL-4	1	4
IL-10	0	4	IP-10	1	4
IL-16	0	4	I-309	1	3
GCSF	0	3	IL-12 p 40	1	3
MIP-1α	0	3	IL-12 p70	1	3
s TNF RI	0	3	IL-15	1	2
IL-1β	0	2	IL-17	1	2
GM-CSF	0	2	TNF-α	1	2
IL-2	0	2	IL-3	1	1
IL-6	0	2	MCP-2	2	3
IL-8	0	2	M-CSF	2	3
IL-11	0	2	TGF-β1	2	3
MCP-1	0	2	IL-7	2	2
TNF-β	0	2	MIP-1σ	2	1
ICAM-1	0	0	IFN-γ	3	4
IL-6sR	0	0	MIG	3	3
MIP-1β	0	0	IL-1α	3	2
RANTES	0	0	IL-13	3	2
sTNF RII	0	0	PDGF-BB	4	2

Levels of 40 different cytokines and chemokines associated with inflammatory responses were analyzed in plasma samples obtained from 4 OMT and 4 sham participants using a cytokine antibody array following the manufacturer's instructions. Plasma samples (pre-treatment and 30 min post-treatment) from OMT and control participants were compared in order to determine changes in the levels of immune molecules due to treatment. doi:10.1371/journal.pone.0090132.t00

   Limitation of these studies associated to a small sample size is that although demographic data was collected was that they were unable to perform a stratified analysis of their results to include gender and age differences. A study involving a larger population would be able to identify differential effects of OMT associated to age and/or gender.an additional limitation of their studies was that only short term effects of OMT (1 h after treatment) were evaluated. Long term effects involving de novo synthesis of cytokines or chemokines were not investigated there and future studies should include them in order to fully understanding the effect of OMT on immune parameters. In this context, it is noteworthy to highlight that they included only healthy individuals in these studies. A study involving individuals suffering inflammatory conditions in which several chemokines and cytokines are being produced at higher levels than normal by activated cells, could help identify the effect of OMT on the regulation of cytokine, chemokine or immune related growth factor production. Indeed, studies on the effect of OMT on elderly populations, which are considered to have low levels of chronic inflammation, might be able to address this issue. Finally, this study focused in changes induced by OMT in which little or no effect from de novo synthesis of immune molecules could be surely observed due to the limited time lapse between treatment and sampling. Even under these stringent conditions we were able

to detect some modification in immune parameters in our studied population. This argues for future studies investigating the effect of OMT at later time points in order to observe effects on the synthesis of circulating immune molecules or the up regulation of activation markers in immune cells.

In closing, the data show for the first time that OMT exerts a modification in the distribution of a particular blood DC population. This was possible due to thorough flow cytometry analysis and multiplex technology. This could help interpret data indicating that OMT can help fight infections, or can increase the efficacy of vaccinations.

Furthermore, this argues for extended immunological studies aiming to determine that OMT might help some conventional anti- infectious therapies. In this context, combination of OMT with conventional therapeutic treatments has the potential of lowering healthcare costs which will result in a great benefit for both the patient and the healthcare system.

In a pilot study designed to determine if OMT influences levels of circulatory pain biomarkers and published by JAOA • Vol 107 • No 9 • Degenhardt et al • Original Contribution , In a prospective, blinded assessment, blood was collected from 20 subjects (10 with chronic low back pain [LBP], 10 controls without chronic LBP) for 5 consecutive days. On day 4, OMT was administered to subjects 1 hour before blood collection. Blood was analyzed for levels of betta-endorphin (b-E), serotonin (5-hydroxytryptamine [5-HT]), 5-hydroxyindoleacetic acid (5-HIAA), anandamide (arachidonoyl ethanol amide [AEA]), and N-palmitoyl ethanolamide (PEA). A daily questionnaire was used to monitor confounding factors, including pain and stress levels, sleep patterns, and substance use.

Increases from baseline in b-E and PEA levels and a decrease in AEA level occurred immediately post treatment. At 24 hours post treatment, similar biomarker changes from baseline were observed. A decrease in stress occurred from baseline to day 5. The change in PEA from baseline to 24 hours post treatment correlated with the corresponding changes in stress. Subgroup analysis showed that subjects with chronic LBP had significantly reduced 5-HIAA levels at 30minutes post treatment (P=.05) and 5-HT levels at 24 hours post treatment (P=.02) when compared with baseline concentrations. The increase in PEA in subjects with chronic LBP at 30 minutes post treatment was two times greater than the increase in control subjects.

Finally, they could reach to this conclusion that Concentrations of several circulatory pain biomarkers were altered after OMT. The degree and duration of these changes were greater in subjects with chronic low back pain than in control subjects without the disorder.

Persistent pain is associated with the production and release of multiple nociceptive (pain) and inflammatory mediators .Although the complexity of the pain-inflammatory process is not fully understood, important roles in this process have previously been suggested for circulatory neurochemical biomarkers, including endocannabinoids, endogenous opioids ,and serotonin. They hypothesized that the concentrations of circulatory biomarkers are influenced by OMT, thus providing objective measures that can be used in future research to better define the underlying mechanisms of OMT. The analgesic properties of plant-derived opiates have been known since ancient times. Endogenous opioids (dynorphins, endorphins, enkephalins) have also

been implicated in pain modulation, both directly and through the placebo response. Opioids act via central and peripheral opiate receptors to produce analgesic effects. In addition, endogenous opioids regulate inflammation through opioid receptors found on immune cells at the site of inflammation.

Pilot studies have been performed to assess a variety of manual treatments on β-endorphin (βE) levels. Although two studies, demonstrated a positive correlation between elevated βE and manual treatments (connective tissue massage and spinal manipulation), other researchers have failed to find such a correlation. As a result of variable experimental methodologies, small sample sizes, and inconsistent outcomes in these studies, firm conclusions cannot be drawn regarding the relationship between manual treatments and endogenous opioid levels.

Serotonin (5-hydroxytryptamine [5-HT]) is a major neurotransmitter component of the inflammatory chemical milieu and a potent stimulant for nociceptive nerve endings in the peripheral nervous system. Serotonin is found in platelets and basophils, where it can be released under conditions of injury, and it acts on more than 15 receptors, of which 5-HT1A, 5-HT2A, 5-HT3, and 5-HT4 have the greatest relevance in nociception. Some studies have shown that serotonin is found at higher concentrations in the blood products of individuals with chronic painful inflammatory conditions, such as fibromyalgia and rheumatoid arthritis. Similar studies involving individuals with chronic low back pain (LBP) have previously not been performed. Furthermore, there have been no published studies evaluating the effects of OMT on serotonin or its metabolic derivative, 5-hydroxy indole acetic acid (5- HIAA), in human subjects. However, Skyba et al have shown in an animal model that mobilization of the knee can induce a release of 5-HT in the spinal cord.

Low back pain is a major healthcare concern, with an incidence rate of 60% to 80% in industrialized countries and etiologic factors that, in approximately 85% of those cases, are considered nonspecific or biomechanical .Osteopathic manipulative treatment, as well as certain other forms of manual therapy, have previously been shown to be beneficial in the treatment of patients with LBP. In the present investigation, they assessed the effects of OMT on five pain biomarkers—βType equation here.E, 5- HT, 5-HIAA, AEA, and PEA—in volunteer subjects with chronic LBP. Because chronic LBP can be explained by pathophysiologic mechanisms involving mechanical and inflammatory mediator-induced abnormalities, they hypothesized that subgroup analysis would allow the consideration of important nuances that are often raised in OMT research, such as the placebo response.

The results of the study showed statistically significant biomarker changes in the overall study population, as well as statistically significant differences between the two subgroups even though the sample size was small. These findings support more rigorous research on the mechanisms of OMT, using a more standardized treatment protocol involving control and light-touch sham treatment groups. Because the data in the present study were skewed, nonparametric statistical analyses were used. For readers not familiar with such statistics, it may appear odd that small changes in the median value can be statistically significant. However, by examining how data for each subject change over time, such variations can illustrate consistent trends of a population or subgroup. Because it is unclear where the biomarkers were formed or how the mechanisms of OMT affected biomarker concentrations, it is possible that small changes in serum biomarker concentrations may reflect larger changes in other tissues. In this study, baseline 5-HT concentrations tended to be lower, while 5-HIAA concentrations were higher in the chronic LBP

group relative to the control group. However, these differences between the subgroups did not attain statistical significance, probably because of large inter subject variability and limited sample size. Relative to baseline and to control levels, levels of 5-HT were reduced at 30minutes and 24 hours post treatment in subjects with chronic LBP. Concentrations of 5-HIAA in subjects with chronic LBP were significantly reduced compared with baseline measures and control subjects at 30minutes post treatment, but not at 24 hours post treatment. The 5-HIAA/5-HT turnover tended to increase in subjects with chronic LBP and decrease in control subjects

Overall, because of the small sample size and large inter subject variability, trends in 5-HT and 5-HIAA levels were not statistically significant. Still, these findings suggest that OMT may reduce peripheral analgesic effects of 5-HT in subjects with chronic LBP by increasing 5-HIAA/5-HT turnover and, thus, decreasing serum 5-HT concentrations. Further studies are necessary to determine if such a relationship exists.

Table 2. Spearman Rank Correlation Coefficients of Change in Self-Reported Pain*by Biomarker Levels for Study Participants With Chronic Low Back Pain (n=10)
Baseline to Post treatment Change

Biomarker	30 min	24h
β-Endorphin	0.10	0.38
Serotonin (5-HT)	0.20	-0.26
Serotonin Metabolite 5-HIAA	0.60	-0.67
5-HIAA/5-HT Turnover	0.07	-0.46
Anandamide	-0.07	-0.34
N-Palmitoyl ethanol amide (PEA)	-0.26	-0.47

* Subjects reported pain on an 11-point numerical rating scale (0=no pain, 10=most severe pain).   Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine

Changes from baseline levels of βE, AEA, and PEA occurred immediately after, as well as 24 hours after, OMT. The data suggest that alterations in levels of circulatory biomarkers were most likely caused by OMT, rather than by changes in potential confounding factors. However, these results are based on small sample sizes and tests with low statistical power. The observed alterations in blood concentration were present, but variable, in both study groups. While encouraging, these results are correlational rather than mechanistic. More rigorously controlled research into the mechanisms of OMT is required before these mechanisms can be adequately hypothesized and tested.

In conclusion, Osteopathic Manipulative Therapy Induces Early Plasma Cytokine Release and Mobilization of a Population of Blood Dendritic Cells. A significant decrease in the proportion of a subpopulation of blood dendritic cells was detected in OMT patients. Significant differences were also detected in the levels of immune molecules such as IL-8, MCP-1, MIP-1a and most

notably, G-CSF. Thus, OMT is able to induce a rapid change in the immunological profile of particular circulating cytokines and leukocytes. This could help interpret data indicating that OMT can help fight infections, or can increase the efficacy of vaccinations.

Also, Osteopathic lymphatic techniques reduces cortisol plasma level in rats after repeated treatments cortisol level was significantly reduced in rats that were treated with lymphatic techniques. In untreated rat group cortisol level was, however, minimally increased. This demonstrates that repeated lymphatic treatments induce reduction in cortisol level, which may be related to an anti-stress effect.

Moreover, Concentrations of several circulatory pain biomarkers were altered after OMT. The degree and duration of these changes were greater in subjects with chronic LBP than in control subjects without the disorder. It has been shown in human patients suffering chronic low back pain, that OMT is able to modify the levels of cytokines such as IL-6 and TNF-α in blood upon repeated treatment. Changes from baseline levels of βE, AEA, and PEA occurs immediately after, as well as 24 hours after, OMT. Published results investigating the relationship between changes in 5-HT and painful inflammatory musculoskeletal conditions, such as fibromyalgia and arthritic joint pain, are complicated. Findings suggest that OMT may reduce peripheral analgesic effects of serotonin 5-HT in subjects with chronic LBP by increasing 5-HIAA/5-HT turnover and, thus, decreasing serum 5-HT concentrations. It is well known that measured biomarkers interact with each other and can produce substantial additive or synergistic analgesic effects. Many reports evidence in humans the positive effect of manipulation, in particular of osteopathic lymphatic techniques in normal as well in pathological conditions. In animals too, lymphatic treatment has been reported to induce changes of lymphatic flux and to mobilize inflammatory mediators. Thus determining positive effects of OMT on the immune system.

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