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RESEARCH LETTER

# Trace element levels in pleural effusions

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## 1 | INTRODUCTION

Pleural effusions are common manifestations of a diverse spectrum of disorders. The fluid contents are complex and not yet fully characterized. It is increasingly recognized that pleural fluid contains biologically active contents, including trace elements. These can contribute to disease pathogenesis, exemplified by our recent finding that manganese is an essential growth factor for *Streptococcus pneumoniae* (a common cause of empyema) in pleural fluid.[1]

Trace elements are fundamental to numerous physiological processes in health and disease states.[2] Prior papers have described selected trace elements in specific pleural conditions.[3-6] In this study, we profiled a large unbiased cohort of well characterized pleural effusions to (a) confirm the presence of six common trace elements (zinc, copper, cobalt, manganese, iron, and molybdenum); and (b) evaluate their relationship with effusion etiology and biochemical characteristics in commonly encountered pleural effusions.

## 2 | METHODS

Pleural fluid samples from patients presenting to our center are routinely collected with informed consent for biobank storage (approved by the Sir Charles Gairdner and Osborne Park Hospitals Human Research Ethics Committee, RGS0000001516). Fluid is transported in sterile containers to the laboratory, centrifuged at 1000*g* for 10 minutes and the supernatant stored at −80°C until analysis.

Clinical etiology for all selected samples was confirmed by clinician review and samples were classified as previously described.[7] Light's criteria were used to define transudative and exudative effusions. Malignant effusions (defined as per recent clinical trials[8]) included those containing cancer cells or were otherwise unexplained exudates in patients with known disseminated malignancy. Benign exudates were nonmalignant (including infective) exudative effusions. Effusions secondary to fluid overload were those with low lactate dehydrogenase (LDH) associated with heart, liver, or renal failure.

"Six of the eight essential trace elements," as defined by Frieden, were readily measurable in our laboratory with validated tests and were chosen for analysis. All biochemical analyses were performed in PathWest Laboratory (QEII Medical Centre, Western Australia), a NATA-accredited facility. Pleural fluid pH analysis was performed using a Radiometer ABL 800 Flex Analyser. Glucose (Hexokinase), protein (Biuret), and LDH (NADH) analyses were performed on the ARCHITECT-c16000 (Abbott). Concentrations of trace elements and quality control material (Clinchek-Urine Control, Recipe Chemicals + Instruments GmbH) were determined simultaneously by inductively-coupled argon-plasma mass-spectrometry using the NexION 300D platform (PerkinElmer). Samples were diluted in a 2% nitric acid diluent containing 0.1% Triton-X100, plus 50 ppm of Internal Standard mix. The acid used for digestion was Suprapur Nitric Acid 65%; Merck Pty Ltd, Darmstadt Germany, Catalog Number 100441. Calibrators were made using a Stock Multi-Element Standard Solution (100 mg/L). Analysis was performed using Kinetic Energy Discrimination mode with helium as the collision gas for dealing with polyatomic spectral interferences.

SPSSv.26 (IBM) was used for statistical analysis. *Correlations* between variables were reported using *Spearman's* coefficient ($r_s$). Intergroup comparisons were performed using Mann-Whitney test or one-way analysis of variance on-ranks with Dunn's posthoc test.

**TABLE 1** Patients and pleural fluid characteristics

| Patient characteristics (n = 105) | | | |
|---|---|---|---|
| Male, n (%) | | 66 | (62.9%) |
| Age, mean (SD) | | 66 | (12.6) |
| Diagnosis, n (%) | Fluid overload | 21 | (20.0%) |
| | Benign exudate | 26 | (24.8%) |
| | MPE | 58 | (55.2%) |
| Pleural effusion characteristics | | Median | IQR |
| Biochemistry | Glucose (mmol/L) | 5.6 | [4.4-6.8] |
| | LDH (IU/L) | 267 | [161-697] |
| | pH | 7.36 | [7.27-7.42] |
| | Protein (g/L) | 38 | [28-45] |
| Trace elements[a] | Zinc (μmol/L) | 6.1 | [3.3-8.7] |
| | Copper (μmol/L) | 9.2 | [5.85-16.2] |
| | Cobalt (nmol/L) | 1.6 | [1.1-2.6] |
| | Manganese (nmol/L) | 10.7 | [5.6-17.5] |
| | Iron (μmol/L) | 7.5 | [3.5-16.8] |
| | Molybdenum (nmol/L) | 7.9 | [5.1-16.9] |

[a]Limit of detection is 3 × SD of the blanks and the limit of quantification is 10 × SD of blanks.

## 3 | RESULTS

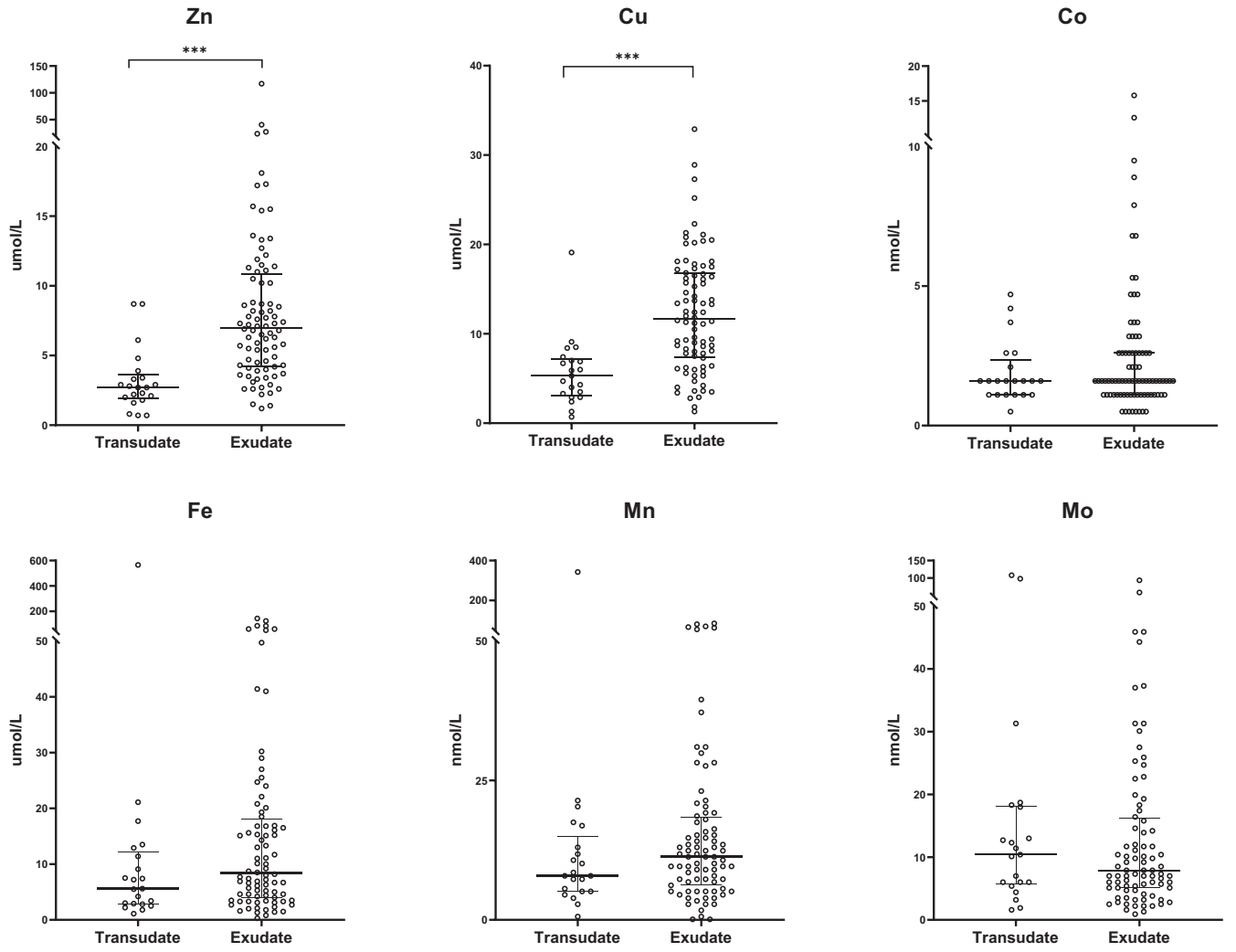
Pleural fluid samples (n = 114) from 105 patients were randomly selected from our Biobank and included effusions from malignancies (n = 58), benign exudates (n = 26), and fluid overload (n = 21) (Table 1).

The six trace elements were present at measurable levels and in biologically relevant concentrations in all pleural fluid samples (Table 1). Overall, their median concentrations in pleural fluid were higher than (especially for Cu and Mo) or within the normal reference range for blood levels. The pleural fluid levels of trace elements were correlated (Table 2); not only between Zn and Cu ($r_s$ = 0.794, $P$ < .001) but also between Zn and Mn ($r_s$ = 0.477), Zn and Fe ($r_s$ = 0.453), Cu and Mn ($r_s$ = 0.437), and Fe and Mn ($r_s$ = 0.728), all $P$ < .001. Overall, pleural fluid trace element levels correlated weakly with the biochemical parameters tested (Table 2).

**TABLE 2** Spearman correlation coefficients between pleural fluid trace elements concentrations and with pleural fluid biochemical parameters

| | | Zn | Cu | Co | Mn | Fe | Mo |
|---|---|---|---|---|---|---|---|
| Zn | μmol/L | - | | | | | |
| Cu | μmol/L | **0.794***** | - | | | | |
| Co | nmol/L | 0.369*** | 0.393*** | - | | | |
| Mn | nmol/L | **0.477***** | **0.437***** | 0.351*** | - | | |
| Fe | μmol/L | **0.453***** | 0.324*** | 0.216* | 0.728*** | - | |
| Mo | nmol/L | 0.301* | 0.300* | 0.229* | 0.246* | 0.079 | - |
| | | **Glucose (mmol/L) n = 110** | | **LDH (U/L) n = 110** | **pH n = 106** | **Protein (g/L) n = 109** | |
| Zn | μmol/L | −0.272** | | **0.527***** | −0.364*** | **0.461***** | |
| Cu | μmol/L | −0.119 | | **0.428***** | −0.267** | **0.573***** | |
| Co | nmol/L | −0.053 | | 0.088 | −0.036 | 0.241* | |
| Mn | nmol/L | −0.101 | | 0.258** | −0.188 | 0.146 | |
| Fe | μmol/L | −0.141 | | 0.235* | −0.123 | 0.100 | |
| Mo | nmol/L | 0.125 | | −0.106 | 0.016 | −0.104 | |

*$P$ < .05;
**$P$ < .01;
***$P$ < .001, $r_s$ > 0.4 in bold.




| | Transudate | | Exudate | | *p* |
|---|---|---|---|---|---|
| | Median | IQR | Median | IQR | |
| Zinc (μmol/L) | 2.7 | 1.9 – 3.7 | 7.0 | 4.2 – 10.9 | <0.001 |
| Copper (μmol/L) | 5.3 | 3.1 – 7.2 | 11.7 | 7.4 – 16.8 | <0.001 |
| Cobalt (nmol/L) | 1.6 | 1.1 – 2.4 | 1.6 | 1.1 – 2.6 | 0.555 |
| Manganese (nmol/L) | 7.9 | 5.1 – 15.0 | 11.3 | 6.4 – 18.5 | 0.214 |
| Iron (μmol/L) | 5.6 | 2.9 – 12.2 | 8.35 | 4.1 – 18.1 | 0.114 |
| Molybdenum (nmol/L) | 10.4 | 5.7 – 18.2 | 7.9 | 5.1 – 16.3 | 0.535 |

**FIGURE 1** Trace element levels in transudative (n = 21) vs exudative (n = 84) pleural effusions. ***$P < .001$

Zn and Cu were significantly higher in exudates than transudates by 2.59- and 2.20-fold, respectively, $P < .001$ (Figure 1). Most trace elements showed statistically significant differences in their concentrations among different etiological groups (Figure 2).

## 4 | DISCUSSION

This study is the largest on trace elements in pleural fluid in a well-phenotyped cohort. All six trace elements studied were present in biologically relevant concentrations in pleural fluid but the concentrations varied among underlying disease groups. These data support future functional research to define the pathobiological effects of these trace elements in pleural diseases.

The pleural cavity is lined by mesothelial cells. In pathological conditions, influx of cancer and inflammatory cells, fibroblasts, and microbes can occur. Knowledge on effects of trace elements on mesothelial cells, though scarce and limited to in vitro data, suggest that they can actively influence a diverse range of biological events. We recently reported that manganese is important for pneumococci proliferation

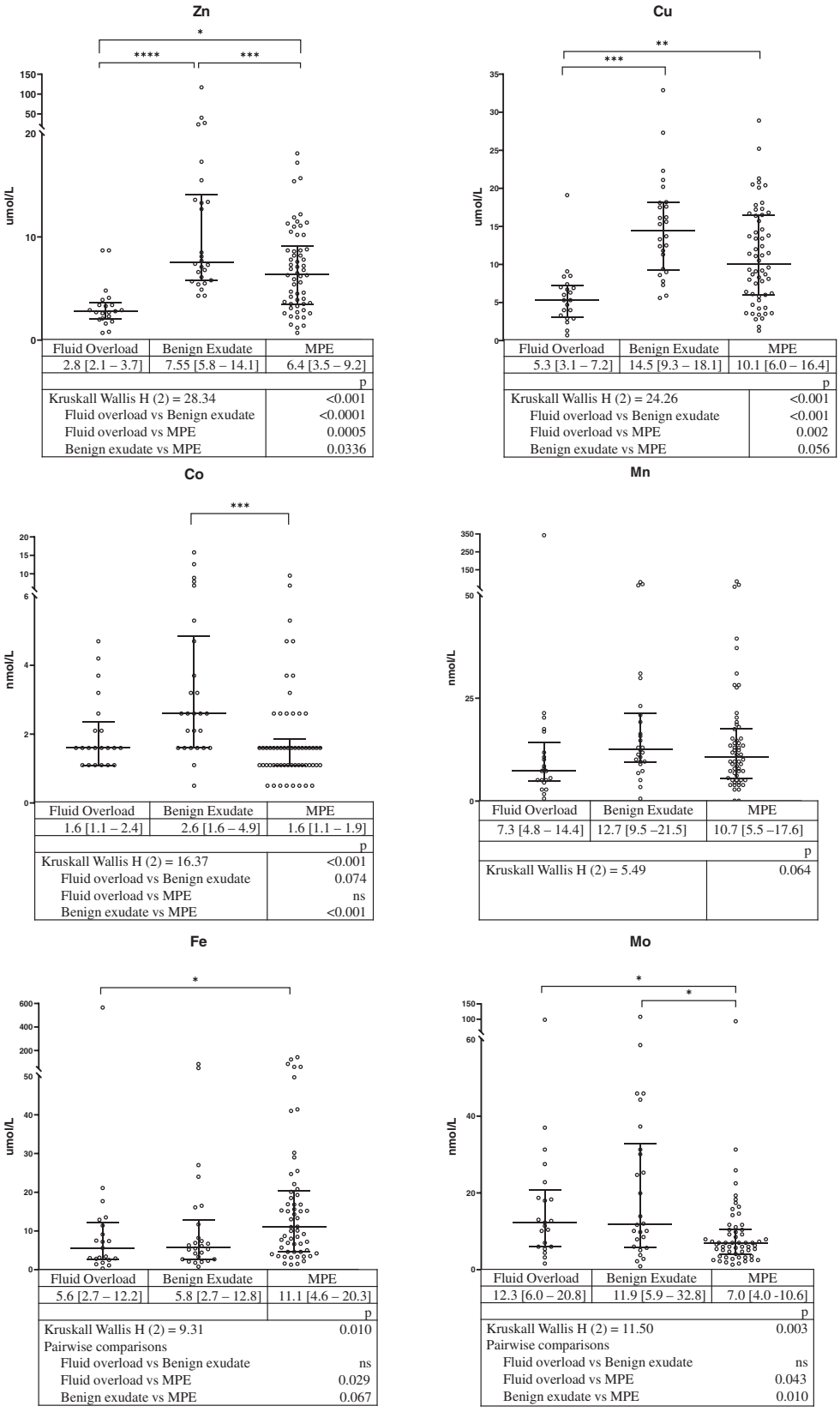



**FIGURE 2** Trace element levels according to pleural effusion groups: fluid overload (n = 21), benign exudates (n = 26) and malignant pleural effusions (MPE) (n = 58). *$P$ < .05; **$P$ < .01; ***$P$ < .001

within pleural effusions.[1] Others found that zinc supplementation blocks the manganese transporter in nonmutant pneumococci by irreversible receptor-binding.[9] Zinc attenuates reactive oxygen species production and inflammation,[10] whereas cobalt and zinc protoporphyrin regulate toll-like receptor-9 expression during infection[11] on mesothelial cells. A recurring outcome in pleural fluid trace element studies is that Zn and Cu are often high in inflamed and infected pleural fluid. We demonstrated that benign exudates contained significantly higher levels

of Zn than MPE and transudates, a finding supported by other studies in the past.[3,4,12] Zn is involved in wound healing and immunity, and functions as an antioxidant. Balkan et al[13] found that serum Zn and protein levels were low in patients with empyema, both increasing in those who received Zn replacement. The reasons for elevated Zn in infection-related effusions are unknown.

Scattered literature suggests a role of trace elements in pleural malignancies. We found higher iron levels in malignant (vs all benign) effusions and higher zinc and copper levels in malignant (vs fluid overload) effusions. Interestingly, iron, as well as overexpression of manganese superoxide dismutase and copper-zinc superoxide dismutase, has been implicated in carcinogenesis of asbestos-related cancers.[10,11,14] Copper-complexed disulfiram has a proposed role against mesothelioma.[15] The role of trace elements in pleural malignancies may deserve further exploration.

We observed that trace element levels were present in high concentrations in pleural fluid (relative to expected blood levels) and their levels were independent of traditional transudate/exudate separation. These observations suggest that transport of trace elements across the pleura involves more than simple diffusion or regulation from Starling's equation. Correlations exist between levels of different trace elements, which may suggest complex coregulatory processes in their biological interactions and transport.

Not surprisingly, trace element levels lack value in separating effusions into benign and malignant.[4-6,16-18] Given the diverse effects of trace elements, it is likely that they are involved in basic mechanisms underscoring different disease processes and are present in significant concentrations across disease states. We found that various trace elements were differentially expressed in different disease states with significantly elevated concentrations. Whether that suggests an active pathogenic role or if they are innocent bystanders will require functional studies.

## 5 | CONCLUSION

Trace elements in pleural disease is an unexplored area. Our data showing their presence in high levels and in differential concentrations across disease states support future investigations of trace elements in pleural diseases.

### CONFLICT OF INTEREST

The authors have no potential conflicts of interest to declare regarding the work involved in this article.

### AUTHOR CONTRIBUTIONS

Conceptualization: Y. C. Gary Lee, Natalia D. Popowicz
Data Curation: Deirdre B. Fitzgerald, Natalia D. Popowicz, John Joseph, Steele C. Butcher, Marie Westcott, Ee Mun Lim, Jenette Creaney
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All authors have read and approved the final version of the manuscript.

Y. C. Gary Lee had full access to all the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

### DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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