

SUPPLEMENT ONLINE

Title: Small particle-size talc is associated with poor outcome and increased inflammation in thoracoscopic pleurodesis.

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Materials and Methods:

Talc particle studies

Scanning electron microscopy. Using coded samples (so that the investigators were blind to the origin and type of talcs), this study was performed at the Instituto de Ciencia de Materiales (Sevilla University, Sevilla, Spain).

Distribution of particle size. It was also studied at the Instituto de Ciencia de Materiales, using laser diffraction technique. *Mineralogical and chemical composition (crystal phases)* of talc was studied using X-rays diffraction and X-ray fluorescence technique, with specification of the main components in each sample.

Biological studies ex vivo (Supplement)

From the pellet we obtained total RNA using QIAmp RNA (Qiagen Diagnostics, GmbH) following the manufacturer's instructions. cDNA was synthesized from 100ng total RNA using Superscript First-Strand (Invitrogen, Carlsbad, CA) following manufacturer's instructions. All PCR reactions were performed on MX3500 Stratagene. Each reaction contained 1x PCR buffer (Brilliant II SYBR Green, Stratagene Cedar Creek, TX, USA), 10 ng cDNA and 10 μ M of each primer. As housekeeping we used RNA 18s.

Cell culture study: Co-incubation of talc with mesothelial cells *in vitro*

Mesothelial cell line Met-5A was obtained from the American Type Culture Collection (ATCC, Rockville, MD), and 10^5 cells were seeded in 12-well plates (Nunc, Denmark) and incubated with $4 \mu\text{g}/\text{cm}^2$ (according to previous reports) [17] of the same two types of talc that were used in patients -as described above- during 24 hours and then IL-8 levels were determined at different time-points in supernatants afterwards.

Cell culture without talc was used as control, and each experiment was repeated five times. Cells were cultured with the recommended conditions and maintained at 37°C and 5% CO_2 , and the supernatants collected and kept frozen for further determinations. Cell viability was measured with Trypan blue dye exclusion (Sigma GmbH, Germany).

Legends for tables and figures (SUPPL. ONLINE):

Table E1. Atomic (%) composition of the two types of talc used in this study.

Figure E1. Relationship between tumor burden found in the pleural cavity at thoracoscopy and outcome of talc pleurodesis. There were significant differences between tumor burden in successful cases and the ones with partial or complete failure ($p=0.001$ and $p=0.02$, respectively).

Figure E2. Correlation between baseline IL-8 in pleural fluid and tumor burden (rated according to reference 15).

Figure E3. Correlation between increments of TNF- α in pleural fluid and serum at 3 hours vs. baseline.

Figure E4. Levels of TAT ($\mu\text{g/ml}$) in serum 24, 48 and 72 hours after intrapleural talc application. Differences were statistically significant between the two types of talc used ($p<0.001$, $p<0.001$ and $p=0.019$, respectively). Points located above box-plots and error bars were outliers in the corresponding group. Outliers in the Small-particle talc were clearly higher than in the Large-particle talc group.

Figure E5A. Kaplan-Meier curve showing overall survival in Small and Large-particle talc groups. No significant differences between groups when considering the whole follow-up period.

Figure E5B. Kaplan-Meier curve showing survival within the first six weeks in Small and Large-particle talc groups. Differences were clearly significant ($p = 0.007$ in the first seven days).

Type of	Si	Mg	Al	Ca	Fe	Na	Ti
Small- particle	30.5	18.8	0.83	0.61	0.15	0.73	<0.1
Large- particle	30.2	19	0.92	0.36	0.48	0.7	<0.1

Table E1 (ONLINE)

Figure E1 (ONLINE)

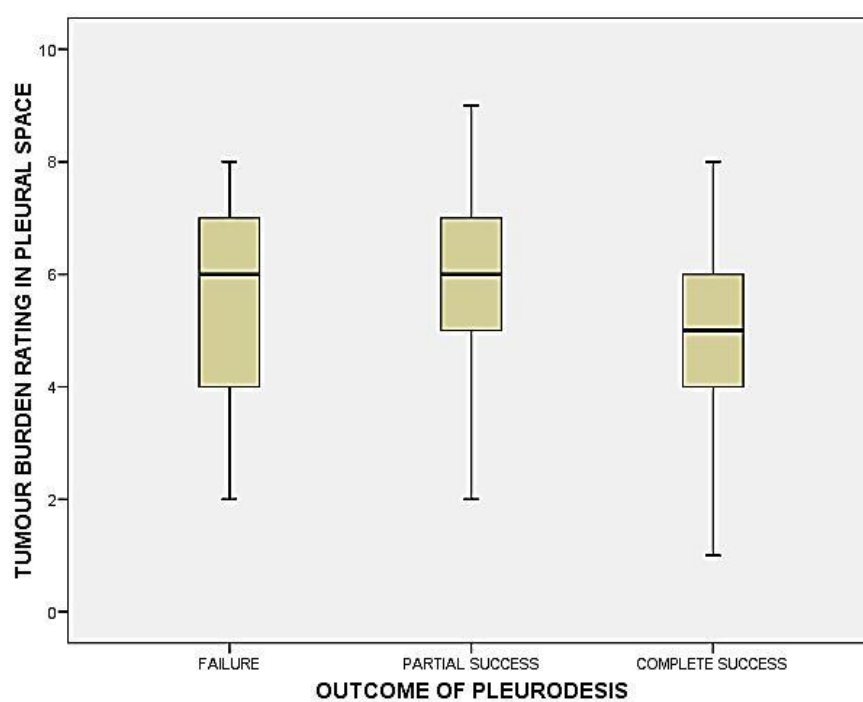


Figure E2 (ONLINE)

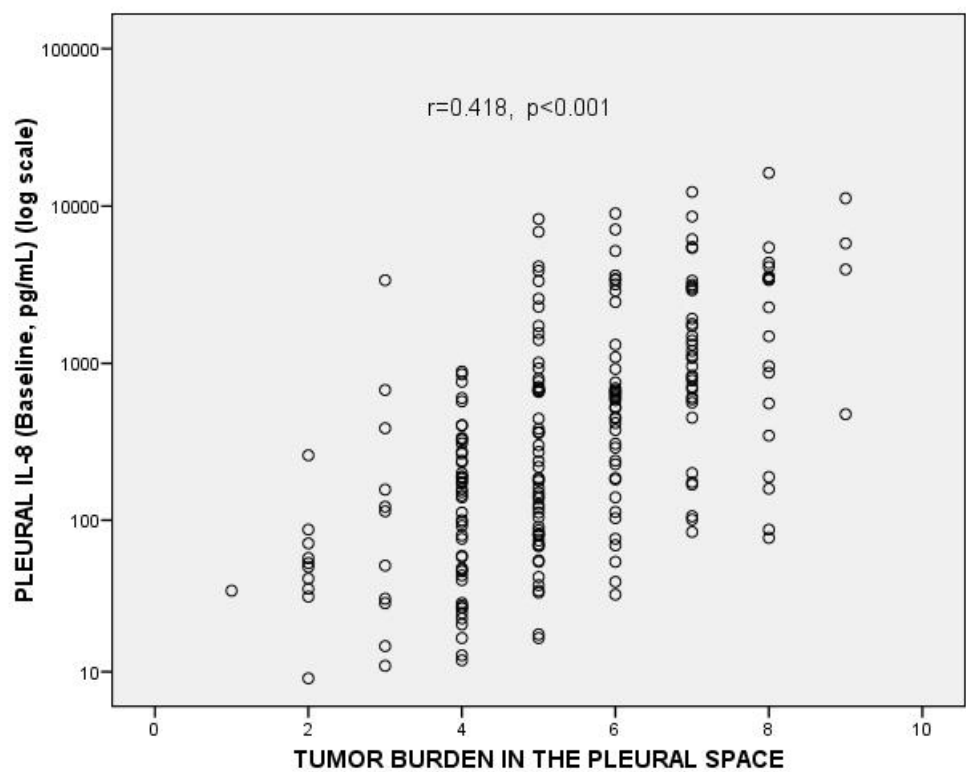


Figure E3 (online)

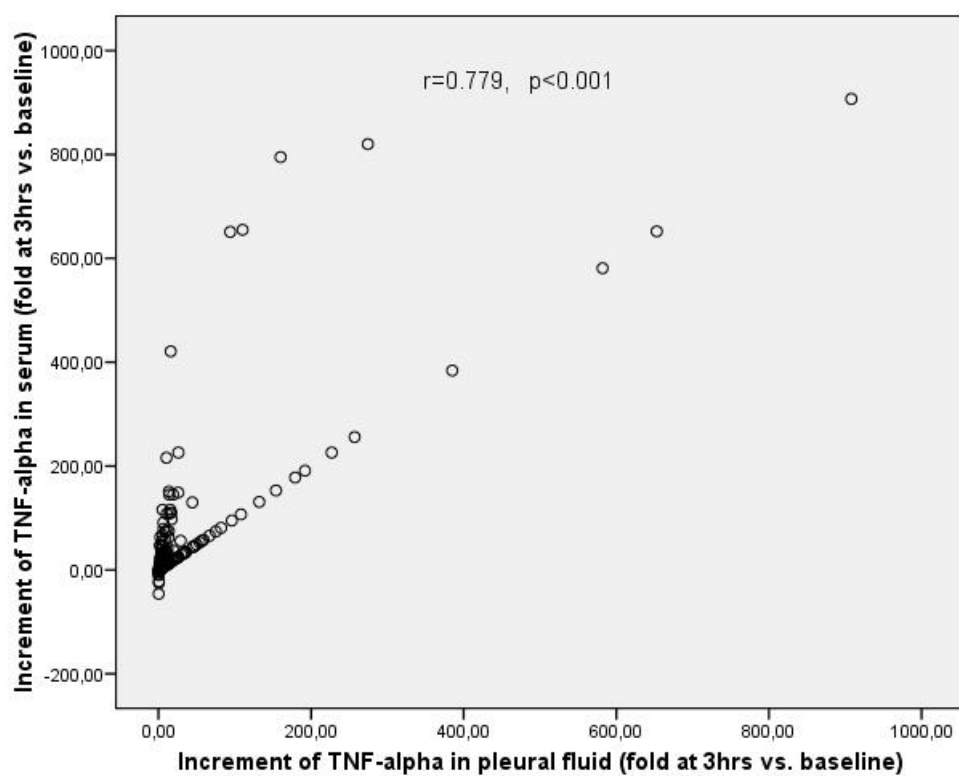


Figure E4 (ONLINE)

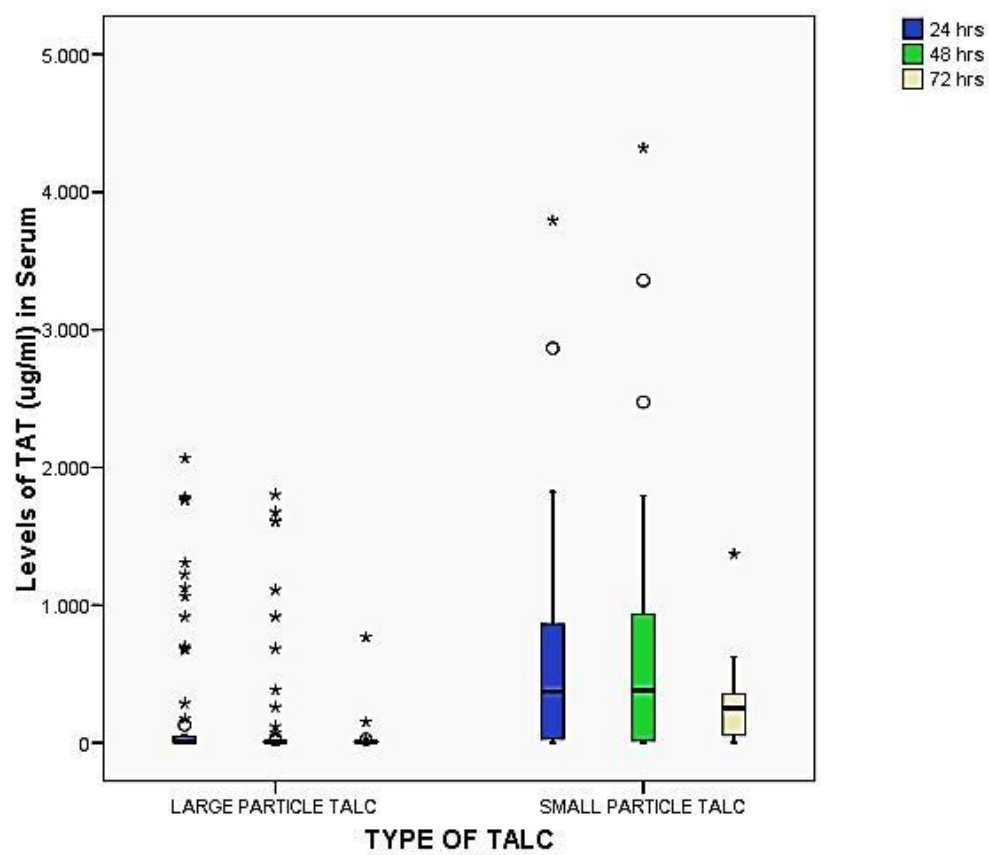


Figure E5A (ONLINE)

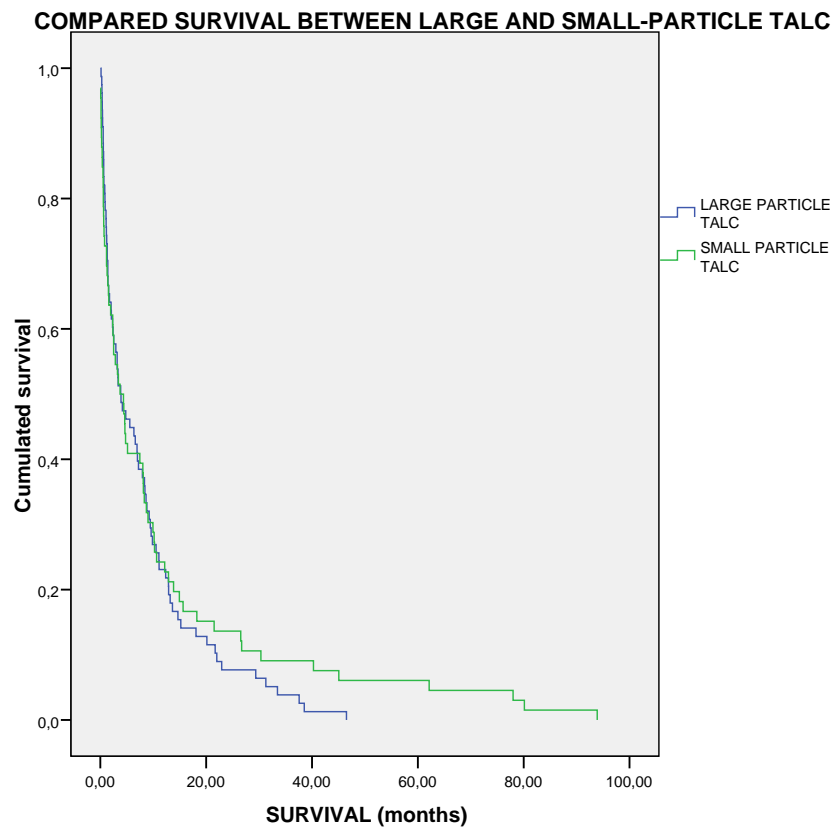


Figure E5B (ONLINE)

