[Year]

Effect of probenecid on synovial fluid ATP levels in CPPD

PROTOCOL AND STATISTICAL ANALYSIS PLAN: NCT02243631

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Study protocol

1. Subjects diagnosed with CPDD according to the McCarty-Ryan criteria are identified through the rheumatolgy clinical practice.
2. Inclusion/Exclusion criteria are met.
3. Subjects are interested in participating in the study and sign an informed consent form and HIPPA.
4. The affected joint is aspirated for 1 cc of synovial fluid, which is immediately placed on ice and sent to the laboratory for analysis of ATP levels
5. The subject is randomized to receive no additional treatment or to receive 2 grams/day of probenecid given in two divided doses.
6. The subject is called daily to monitor symptoms in the affected joint and any side effects.
7. The subject is seen again by the study team on day 6. Another 1 ml sample of synovial fluid is removed from the affected joint and sent to the laboratory for ATP analysis. The subject is then offered an intra-articular corticosteroid injection in the affected joint.
8. This completes the study.

ATP measurement in Synovial Fluid

<u>Basal Salt Solution pH 7.5</u>

130 mM NaCl
5 mM KCl
1.5 mM $CaCl_2$
1 mM $MgCl_2$
5 mM Glucose
0.1% BSA

Mix and then check pH, adjust with NaOH

ATP substrate (FL-AAM from Sigma): Add 5 mL water to vial according to manufacturer's directions.

For standard curve: dilute ATP substrate in salt solution in a ratio of 40% substrate and 60% salt solution. Add 25 μl/well for the standard curve.

Standard curve uses the following concentrations of ATP.
(1 nM, 2.5 nM, 5 nM, 10 nM, 25 nM, 50 nM, 100 nM)

100 μl of salt solution is added to the first well to serve as the zero.
100 μl of each of the concentrations of ATP

To Measure the synovial fluid (SF) : 100 μl of SF (fresh or in citrate) is added to each of 3 wells and then 4 μl of the stock ATP substrate is added to the wells. Tap the plate on the counter to mix then read immediately in a plate reader set for luminescence.

N.B. Synovial fluid needs to be measured within 1 hour of collection to avoid loss of ATP.

Statistical Analysis Plan

The primary outcome will be ATP levels measured from extracted synovial fluid. The two treatment groups include those who received probenecid and those with no intervention. A baseline measurement will be taken at the beginning of the study period and another measurement taken at 7 days. Each participant will serve as their own control. A delta will be calculated (7-day ATP level – baseline ATP level) for each participant and a non-parametric Mann-Whitney t-test will be performed. ATP levels commonly require non-parametric analysis as it tends not to be normally distributed. The mean change between baseline and the 7-day timepoint along with the corresponding 95% confidence interval will be presented.

A Wilcoxon matched pairs t-test will be used to assess whether the intervention produced a statistical difference between the two timepoints with probenecid treatment.