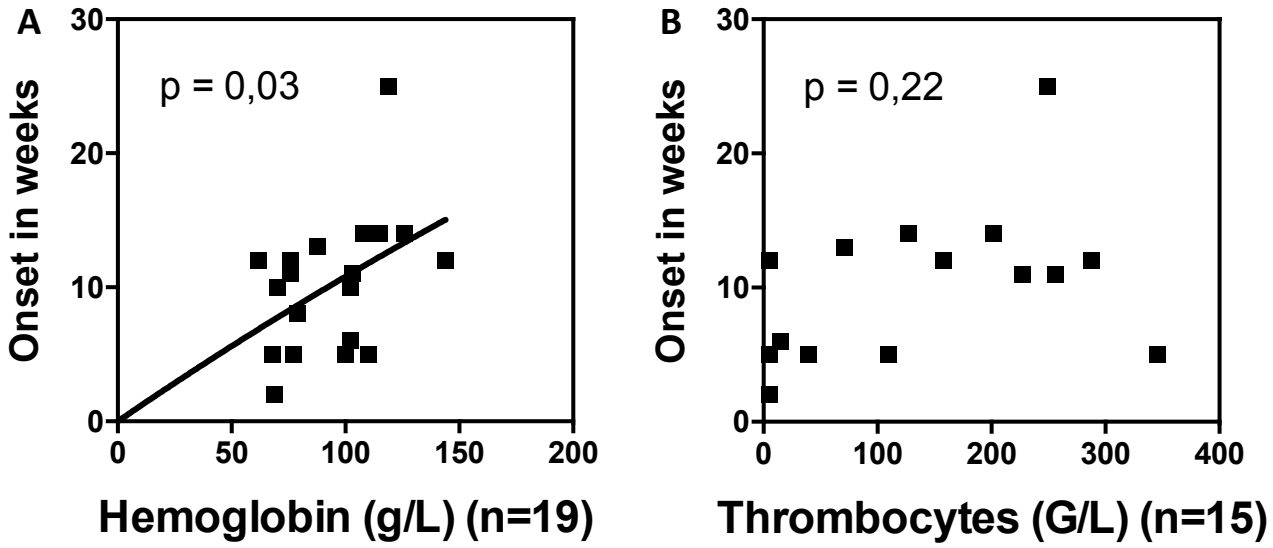Additional file 1