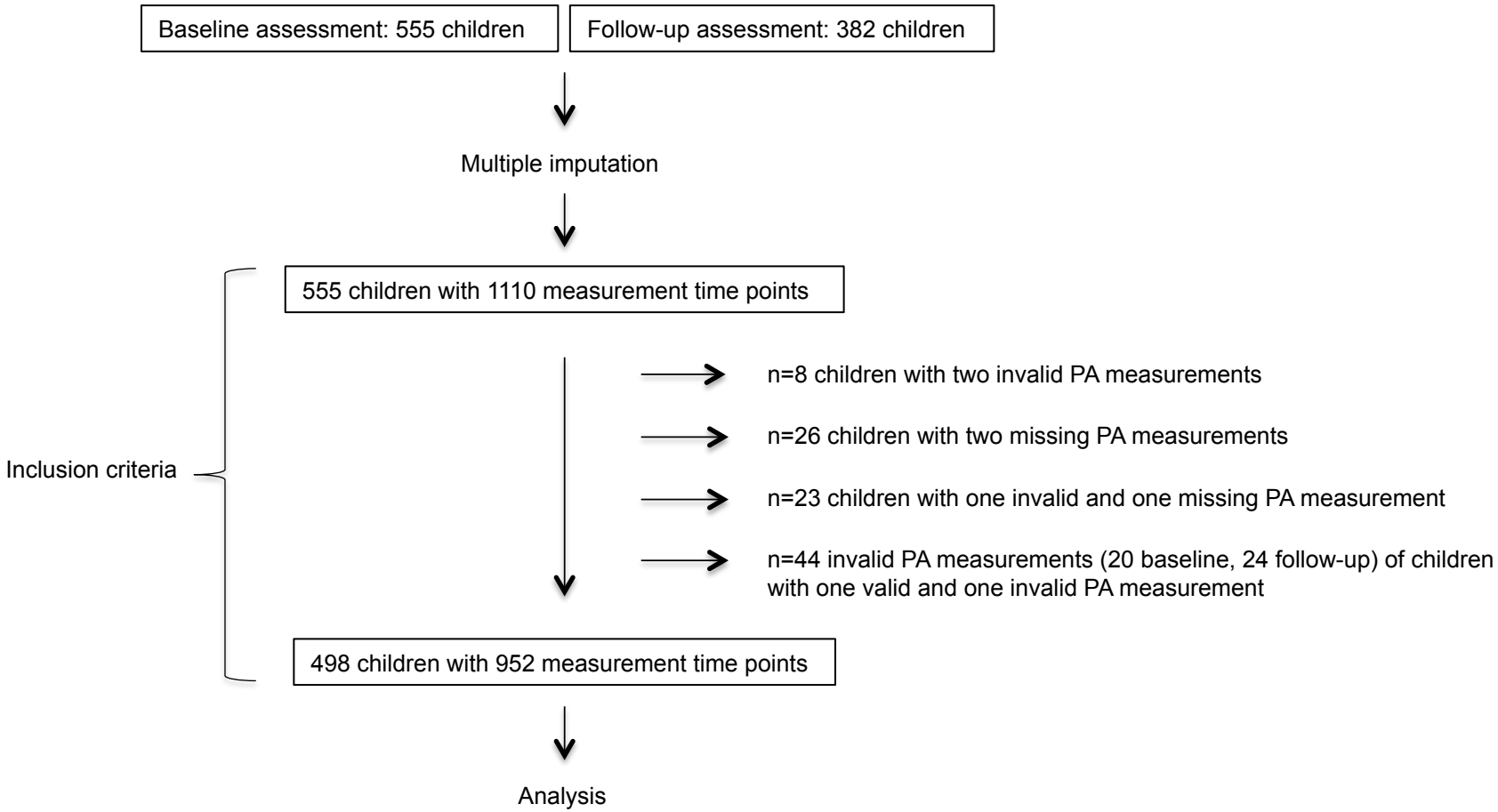Additional file 2. Flow Chart