Supplementary Data

Immunohistochemistry staining. Clinical specimens were fixed in formalin, embedded in paraffin and cut into 4-μm sections. In brief, paraffin was removed from the tissue using xylol and dehydrated in a graded series of ethyl alcohol concentrations. Antigens were retrieved by boiling with sodium citrate solution (pH 6) for 20 min in a microwave. After washing in 0.05% Tween-80 in PBS, the slides were incubated in methanol for 15 min and blocked with PBS containing 10% horse serum and 3% bovine serum albumin (ST025; Beyotime Biotechnology) for 30 min. The sections were incubated with the appropriate antibody [D2-40 (ZM-0465, 1:100 diluted), cytokeratin (ZM-0067, 1:100 diluted); Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.] and transferred to a 4˚C refrigerator for overnight incubation. After washing, the slides were incubated with the appropriate conjugated secondary antibody (PV-9000; ready to use; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.) for 30 min at 37˚C. Finally, the slides were dehydrated through graded ethanols, cleared in xylene and mounted with a coverslip using mounting medium. The slides were analyzed using a microscopic imaging analysis system (IX71; Olympus Ltd.).

H&E staining. Tissue sections were first dewaxed in xylene and rehydrated through a graded ethanol series to distilled water. The slides were then stained with hematoxylin for 5 mins to color the nuclei blue-black, rinsed in tap water, differentiated briefly in acid alcohol (1-2 sec) and washed in running tap water for 5 mins. Subsequently, the sections were incubated with eosin for 3 mins to stain the cytoplasm and extracellular matrix pink, followed by a quick rinse in distilled water. Finally, the slides are dehydrated through graded ethanol, cleared in xylene and mounted with a coverslip using mounting medium. The slides were analyzed using a microscopic imaging analysis system (IX71; Olympus Ltd.).

Figure S1. Schematic flow diagram illustrating the diagnostic approach to large cystic abdominopelvic masses in adolescent patients. The schematic outlines key steps in clinical and image assessment, differential diagnosis and surgical decision-making. It emphasizes the importance of considering non-adnexal origins, recognizing imaging pitfalls and confirming the diagnosis through surgical and pathological evaluation.